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FINAL REPORT
PROJECT B-444

ELECTROMAGNETIC RADIATION STUDY OF TECHNIQUES FOR THAWING FROZEN ORGANS

By

H. A. Ecker, F. L. Cain, and E. C. Burdette

GRANT NO. ENG-74-22318

AUGUST 1976

Prepared for

NATIONAL SCIENCE FOUNDATION
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FOREWORD

The research on this program was performed by personnel of the Applied Engineering Laboratory at the Georgia Institute of Technology, Atlanta, Georgia 30332. Dr. H. A. Ecker served as the Principal Investigator. This program, which was sponsored by the National Science Foundation, Washington D.C. 20550 under Grant No. ENG-74-22318, was designated by Georgia Tech as Project B-444. This Final Report covers the work which was performed during the period from March 1975 through March 1976.

This work was made possible through the combined efforts of many people at the National Science Foundation, at the Medical College of Georgia, and at the Georgia Institute of Technology. The authors would especially like to thank Dr. N. Caplan at NSF, Dr. A. M. Karow, Jr. at the Medical College of Georgia, and Mr. M. L. Studwell and Mr. V. E. Bernard at Georgia Tech all of whom contributed significantly to the success of this research program.

Respectfully submitted,

H. Allen Ecker
Principal Investigator

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SECTION I

INTRODUCTION

The development of electromagnetic radiation techniques for thawing frozen organs is a key feature in establishing a procedure for successful transplantations of organs obtained from a frozen organ bank. At the present time, kidney transplantations occur very infrequently because properly matched donor kidneys are not available at the time of need. In order to establish an organ bank to remedy this situation, the organs must first be frozen for preservation and then thawed at the time of need. Failure to accomplish the prescribed conditions of rapid and uniform thawing results in excessive tissue damage. Until now, very little progress to thaw the organs has been accomplished because of the limitations of the available thawing techniques. However, new engineering techniques currently are being applied to solve medical/biological problems associated with developing methodologies for long-term preservation and subsequent thawing of deeply-frozen human kidneys. Therefore, the application of controlled electromagnetic radiation offers great hope in this area, as the very encouraging results of electromagnetic thawing studies at Georgia Tech show.

The overall objective of this research program is to study non-ionizing electromagnetic radiation techniques for thawing frozen organs. The success of electromagnetic radiation to rapidly and uniformly thaw frozen organs, which is currently the only promising method, depends on the following factors: (1) the frequency of the radiation, (2) the modulation of the radiation, (3) the applied power level, (4) the size and shape of the organ, (5) the dielectric constant of the organ tissue, (6) the loss tangent of the organ,

(7) the level of the cryoprotectant drug in the organ, (8) the thermal state of the organ, and (9) the doping of the organ with a recoverable material. The interrelationships and significance of these factors must be fully recognized in order to achieve rapid and uniform thawing of large organs.

Engineering tasks which involved the above factors were performed and successfully concluded during this one-year program, and the initial engineering basis for an electromagnetic illumination system for use in thawing cryopreserved kidneys for transplantation has been established. One of the keys to the successful cryopreservation of cells, tissues, and organs is the ability to efficiently thaw the biological material without damage. Accurate control of the rate and uniformity of the thawing process in addition to knowledge of the effects of any cryoprotective agents employed during freezing are requirements for success. Results have shown that the illumination patterns and power level of the electromagnetic radiation must be carefully controlled to obtain rapid uniform thawing. Moreover, an accurate knowledge of the electrical properties of the materials as functions of both temperature and cryoprotectant level is required to exercise appropriate control on the radiation. Electromagnetic thawing of canine kidneys has been shown to be feasible, and the current results indicate that with additional engineering studies, a practical dual-frequency system for thawing kidneys comparable in size to human kidneys would be possible.

SECTION II

RESEARCH PUBLICATIONS

Major results of this research have been widely disseminated to the scientific community. Three oral presentations were given at 1976 IEEE Southeastern Conference, at the IEEE 1976 International Symposium on Electromagnetic Compatibility, and at the Thirteenth Annual Meeting of the Society for Cryobiology in April, July, and August, respectively, of this year. In addition, the papers corresponding to these oral presentations either have been or are scheduled to be published in the open literature by the respective sponsoring technical societies. The following tabulation gives the pertinent information.

Burdette, E.C. and Studwell, M.L., "Dielectric Measurements of Cryogenically Preserved Blood and Tissue", Proceedings of the 1976 IEEE Southeastern Conference and Exhibit, Clemson, S.C., April 1976, pp. 379-381.

Ecker, H.A., Burdette, E.C. and Cain, F.L., "Simultaneous Microwave and HF Thawing of Cryogenically Preserved Canine Kidneys", Record of the IEEE 1976 International Symposium on Electromagnetic Compatibility, Washington, D.C., July 1976, pp. 226-230.

Burdette, E.C., Karow, A.M., and Cain, F.L., "Effects of Dimethyl Sulfoxide on Electromagnetic Thawing of Cryopreserved Kidneys", Journal of Cryobiology, Washington, D.C. , August 1976, to be published.

The work reported in the second publication above was wholly supported by NSF under Grant No. ENG-74-22318, whereas the work reported in the remaining two was partly supported by NSF under this research grant.

SECTION III

BACKGROUND

One of the key factors contributing to the successful cryopreservation of whole organs is the ability to thaw the biological material without damage. The old conventional procedure for thawing frozen tissue consists of placing the biomaterial in a 37°C water bath and agitating the biomaterial until it is completely thawed. This method, although most often used, possesses many of the least desirable characteristics. The tissue, which is subjected to an extreme thermal gradient, reaches equilibrium at a final temperature near that of the 37°C water bath. Three major problems associated with this procedure are cell damage due to (1) mechanical stress, (2) chemical problems involving the transport of electrolytes, nonelectrolytes, volatiles, and water across cell membranes, and (3) increasing cryoprotectant toxicity with increasing temperature.

It has been reported that rapid thawing (in excess of 20°C/min) helps to eliminate both the physical and chemical problems [1]. In fact, with the exception of mouse embryos, which should be warmed no faster than 25°C/min [2], many investigators believe it is impossible to warm too quickly. If warming is slow, recrystallization can occur. This is obviously disastrous to cells if very small innocuous intracellular crystals undergo recrystallization. In several investigations, it has been reported that intracellular microcrystals can be sometimes innocuous and that cells can survive freezing as long as the crystals remain small [3,4].

If the duration of thawing is prolonged, chemical injury to cells will occur as a result of exposure to high concentrations of solutes at relatively

high temperatures. These high concentrations are created during the freezing process; as liquid/water is converted to ice, the concentration of solutes rises until the eutectic temperature is reached. But this process is not so injurious during freezing because the temperature is being continually reduced, inhibiting any adverse effects which would result from increases in the level of concentration. During thawing, chemical injury can oocur because the temperature is not only rising, but also the phase transition process is likely to be delayed when relatively large masses of tissues are involved.

A warming rate greater than 20°C/min is desirable, but is technically difficult to achieve with a large mass of tissue such as the canine kidney. In reports of survival of frozen canine kidneys after transplantation, the warming rate has been in excess of 3°C/min, and Dietzman reported that rates exceeding 70°C/min were ncessary to obtain viable functioning kidneys. Rapid thawing is no problem with thin sheets of tissues weighing less than 5 grams, but large organs are 10 to 20 times heavier than this. Thus skin and cornea (tissues which do survive freezing and thawing) can be warmed fast enough to enable survival whereas kidneys, hearts, and livers cannot. The problem with large organs relates to the difficulty of pumping 80 calories/gram of tissue/°C to achieve a thawing rate in excess of 10°C/ minute. Thawing becomes increasingly difficult as more and more ice becomes liquid, since liquid water is a thermal insulator. If a frozen canine kidney is placed in perfusate solution at 37°C, the temperature of the surface rises sharply to 37°C while the core slowly equilibrates (45 to 90 minutes) at the melting point until sufficient heat can be transmitted through the growing layers of insulation to provide the latent heat of fusion. This delay

in thawing has been repeatedly demonstrated by temperature recordings in model systems and in actual organs (where severe tissue damage resulted) [1].

Recently, researchers have identified electromagnetic (EM) heating techniques as potential methods for solving the problem of rapid, uniform thawing of cells and tissues [5]. Early EM research attempts to thaw several types of whole organs with commercial microwave ovens usually produced very poor results [5,6]. Producing the proper electromagnetic illumination for successfully heating cells, tissues, or whole organs is an extremely complicated process; not only is the determination of the field incident on an organ in a given microwave oven virtually impossible, but the field configurations generally change from one oven type to another. Even the most sophisticated electromagnetic heating techniques involving resonant cavities that have been investigated by several researchers for use with canine kidneys have met with serious difficulties [7]. The presence and the level of cryoprotectant drugs may significantly affect the dielectric properties of the frozen cells or organs, and hence, affect the electromagnetic thawing rate. Therefore, not only must an acceptable EM radiation thawing technique be established, but also the technique must account for the possibility of varying electrical properties due to the presence of cryoprotectants and changing temperature as thawing occurs.

At Georgia Tech, the use of controllable electromagnetic radiation for rapid, uniform thawing of rabbit kidneys and frozen granulocytes is being investigated [8-11]. A significant advantage in EM thawing over any other potential rapid thawing technique is its ability to simultaneously penetrate completely through the biological material and thus uniformly

thaw an entire kidney rather than thawing from the surface to the inside of the organ. The EM thawing techniques developed during this research program have produced encouraging results which indicate that with an adequate allocation of resources, long term cryopreservation of whole organs could becomes a reality. To provide an insight into problems associated with successfully thawing frozen cells or tissues, pertinent aspects of the theory of electromagnetic heating are described in the following paragraphs.

The interaction of electrical, thermal, and biological properties of cells or tissues must be considered before any instrumentation for successful thawing can be implemented. Figure 1 depicts the interaction of parameters involved in the thawing process. Basic properties of prime importance associated with the process of thawing by EM radiation are the dielectric constant, loss tangent, sepcific heat, and thermal conductivity. In general, each of these properties is a function of temperature; the electrical properties (dielectric constant and loss tangent) are, in addition to temperature, functions of the frequency of EM radiation as indicated in Figure 1.

Biological material consists of three dielectric components that exhibit different relaxation frequencies; a relaxation frequency [12,13] characterizes the rate at which a system of dipoles (associated with molecules) approaches a new equilibrium distribution in response to an electric field. These three components are (1) water, as free interstitial and body fluids, relaxation frequency 20 GHz; (2) bound water, mostly inside cells, relaxation frequency 1-10 MHz; (3) proteins and amino acids, relaxation frequency 100 MHz. The existence of a relaxation frequency is related to the fact that reorientation of dipoles (water is a polar substance) cannot occur instantaneously

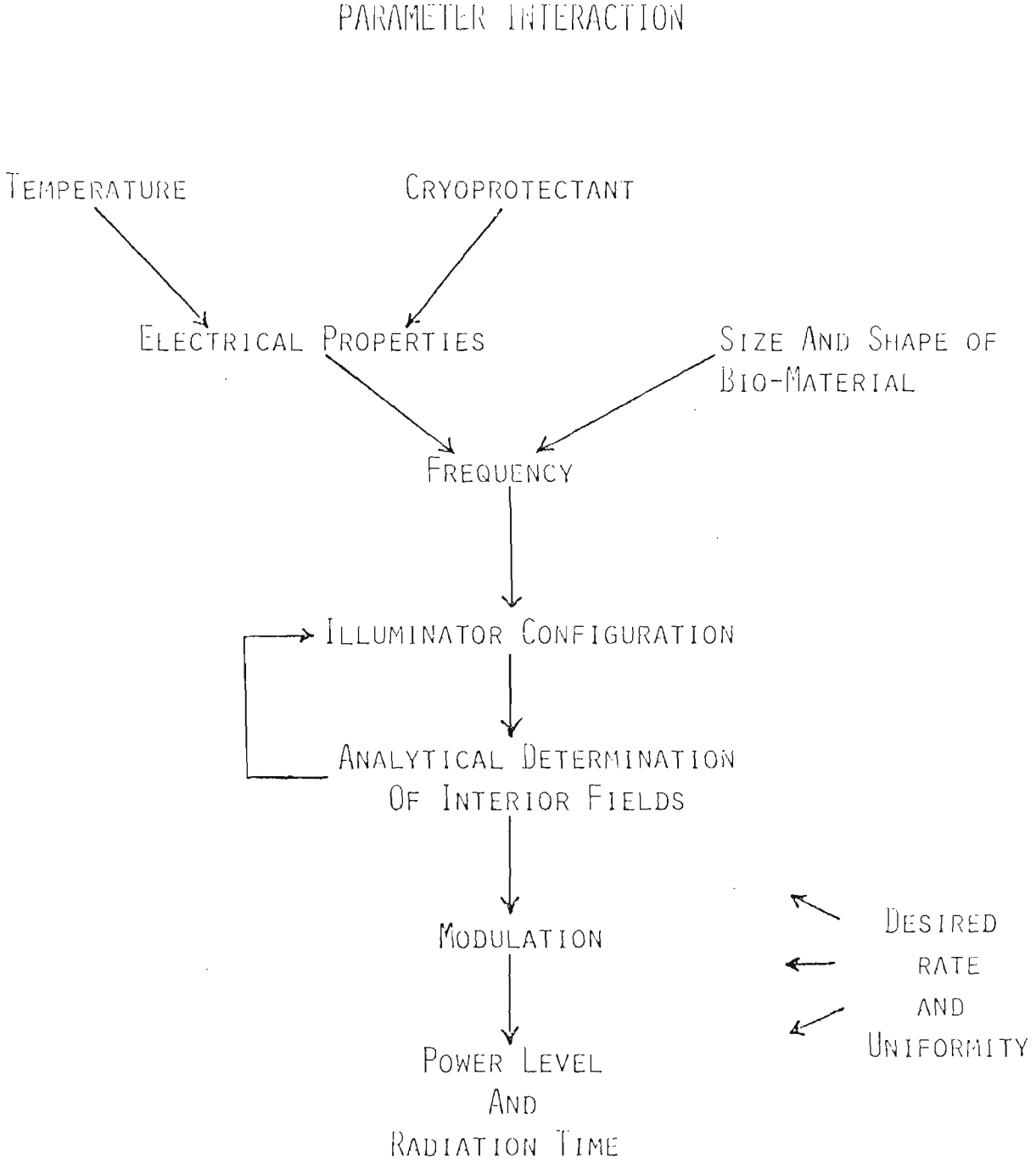


Figure 1. Interaction of critical parameters.

in response to changes in electrical field. Thus, because there are different relaxation frequencies at which high losses occur, different components of a biological system are heated at different rates.

In many media, there also exists a second mechanism of dielectric heating which is associated with quantized transitions from one energy level to another in an atom or molecule [14]. When the applied frequency is resonant, a sharp absorption peak occurs resulting in heating of the material.

The losses resulting from the interaction of an electric field and a dielectric material are generally described from a macroscopic viewpoint in terms of the dielectric loss tangent, which is the ratio of the imaginary part to the real part of the complex permittivity. The conductivity of the material is related to the loss tangent by

$$\tan\delta = \frac{\sigma}{\omega\varepsilon_o K} \quad , \tag{1}$$

where σ = conductivity (mho/m),

ω = frequency of radiation,

ε_o = permittivity of free space,

$\tan\delta$ = loss tangent, and

K = relative dielectric constant.

In addition, the magnitude of the losses depends on the strength of the electric field coupled into the material. The magnitude of the electric field coupled into the material, as well as its variation within the material, are functions of both real and imaginary parts of the complex permittivity. Because biological materials contain a large percentage of water, there is an abrupt

change in the state of a material during the thawing process since the materials possess icelike characteristics in the frozen state. Consequently, the thermal properties of the material are different. Cryoprotectant additives such as DMSO or glycerol also have abrupt changes in relative dielectric constant and conductivity at temperatures near the phase change of water.

The knowledge of the relative dielectric constant and the loss tangent of a lossy dielectric material allows the determination of the penetration depth $1/\alpha$ and power absorbed per unit volume P in the medium. The penetration depth is that distance in which the incident electromagnetic fields have been attenuated to 1/e or 0.368 of their initial value. The penetration depth can be expressed in terms of the dielectric constant and loss tangent of the medium as [15]

$$1/\alpha = \frac{3.0 \times 10^{10}}{\omega\sqrt{\frac{K}{2}}\sqrt{\sqrt{1 + \tan^2\delta} - 1}} \quad \text{cm}, \tag{2}$$

where the symbols are as defined previously. If the penetration depth is greater than the thickness of the biological sample to be heated, the magnitude of the electric field in the tissue is nearly constant. In this case, the amount of electromagnetic power absorbed per unit volume of lossy dielectric medium can be written in terms of the incident electric field E_o as

$$P = \omega\varepsilon_o K \tan\delta \left[\frac{E_o^2}{2}\right] \quad \frac{\text{watts}}{\text{cm}^3}. \tag{3}$$

The electrical characteristics of frozen and thawed materials can vary significantly. For example, pure ice at a temperature of -12°C and a frequency of 3 GHz has a dielectric constant of 3.2 and loss tangent of 0.009. Pure water has a dielectric constant of 76.7 and loss tangent of 0.157 at the same frequency [12]. Therefore, the change in dielectric constant during the phase transition from ice to water is greater than 20 to 1, and the change in loss tangent is greater than 15 to 1. Using the above values as the ice changes to water, the resultant increase in power absorbed could be greater than 300 to 1.

These drastically different electrical characteristics of ice and water produce both favorable and unfavorable effects. As indicated above, because of the relatively low dielectric constant and loss tangent of ice, the electric fields in a homogeneous biological sample whose properties are similar to those of ice will tend to be uniform, and hence a reasonably uniform heating throughout the material is expected. However, if a change in state occurs in a localized region, the loss tangent of that particular region will increase, and more heat will be absorbed in that region than in the portion of the material that is still frozen. This phenomenon is generally called thermal runaway and must be controlled to prevent excessive localized heating and cell damage. To minimize this effect, the electrical characteristics of the electromagnetic applicator and the sample being irradiated should be matched to maximize heating of frozen cells or tissue and minimize heating of thawed cells or tissue.

The thawing rate also depends on the thermal properties of the material. For example, the thermal conductivity of ice is higher than the thermal

conductivity of water. Thus, in conventional water-bath heating or non-uniform electromagnetic heating techniques, the outer surface thaws prematurely, and the localized liquid area insulates any unthawed region. This thermal insulating effect is enhanced for the case of electromagnetic heating because the liquid material will also have a much higher loss tangent than the solid material, and therefore, act as a barrier to electromagnetic radiation, thus accentuating the thermal runaway problem. This latter effect can be controlled through the use of a feedback control system which automatically reduces the incident power level at a rate proportional to the rate at which the relative dielectric constant and loss tangent are increasing. Therefore, the illuminator must not only efficiently couple energy to the cells in the frozen state, but must also sense changes in the electrical properties of the cells to control both the power level and exposure time.

SECTION IV

ENGINEERING STUDIES INVOLVING EM THAWING TECHNIQUES

The various factors and techniques necessary for successful electromagnetic (EM) thawing of deeply-frozen organs are presented and discussed. These investigations include (1) determination of electrical properties of tissues, (2) analytical modelling of EM internal field distributions in kidneys, (3) designs for EM thawing systems, and (4) results for thawed kidneys. Typical results for these investigations are summarized in the following subsections.

A. Determination of Electrical Properties

The effectiveness of electromagnetic radiation for direct heating of cryopreserved kidneys is critically dependent upon the electrical properties (dielectric constant and loss tangent) of the tissue. These properties are functions of (1) the frequency of radiation, (2) the temperature of the organ, (3) the phase state of the organ, and (4) the cryoprotectant level.

The electrical properties of biological materials are conventionally determined by either measuring the reflection coefficient or changing the sample length. Repeated slicing of fresh and therefore soft tissue within the required accuracy of approximately 0.05 mm for employing the method involving a change of the sample length is very difficult, if not often impossible. Therefore, standard short-circuited transmission line techniques were used to determine the electrical properties of fresh kidney tissue at 2450 MHz. A system which employed a waveguide slotted-section and probe attached to a short-circuited waveguide holder [12,16] was utilized for determining the reflection coefficient of biological samples. The electrical

properties of a sample were determined from measurements of the width and shift of the nulls in the standing wave pattern produced in the short-circuited waveguide, with and without a sample placed against the short as shown in Figure 2. To check the accuracy of the technique and instrumentation, initial measurements of several standard materials including de-ionized water, methanol, and silica were performed. The results of these measurements, which are presented in Table I, indicate very good agreement with the data obtained from reference material [12,17].

Once the measurement accuracy of the system was established, measurements of the electrical properties of the cryoprotective agent Dimethyl Sulfoxide (DMSO) in a kidney perfusate solution were performed. The results shown in Table II indicate a difference in relative dielectric constant and conductivity equal to or greater than an order of magnitude between the frozen and thawed states. Within a particular phase state, the DMSO concentration in the perfusate solution did not produce a drastic change, but changes in both conductivity and relative dielectric constant are discernable. Other than the change in dielectric properties with changes of phase state, the most significant effect is the change in conductivity and loss tangent in the frozen state when DMSO is added to the kidney perfusate solution.

Calculations of the dielectric properties of kidney tissues excised from various portions of both canine and rabbit kidneys indicated that the waveguide measurement technique was very sensitive to variations in the smoothness of the sample surface as well as the sample thickness. As the sample placed against the short was decreased in thickness, large variations in the calculated dielectric properties were observed for the same tissue type. Also, significant variations in the results were observed

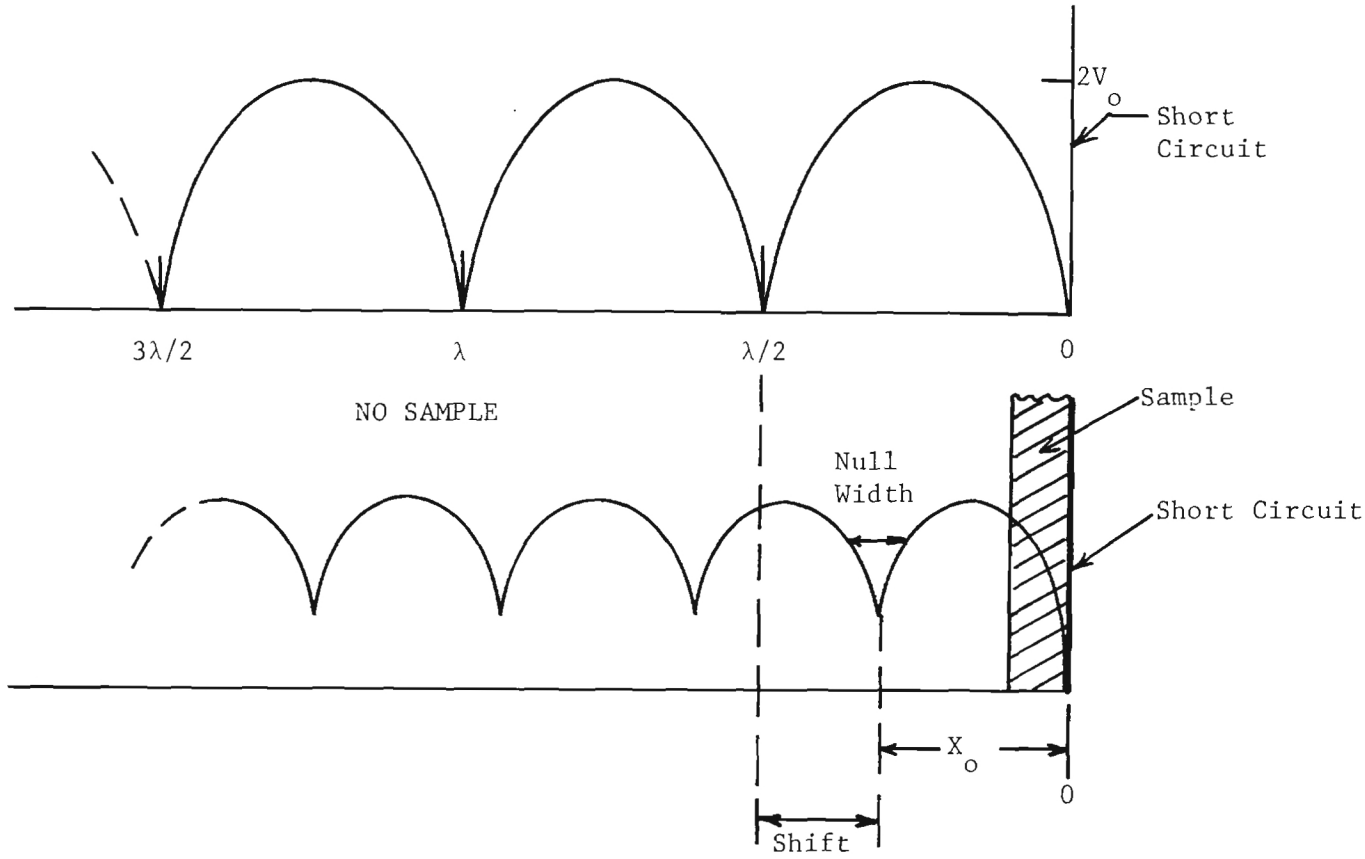


Figure 2. Measurement of dielectric properties from changes in standing wave pattern in short-circuited waveguide.

TABLE I

ELECTRICAL PROPERTIES OF SAMPLE STANDARDS AT 2450 MHz
MEASURED USING WAVEGUIDE MEASUREMENT SYSTEM

Sample	Dielectric Constant	Loss Tangent	Conductivity (mho/m)
Deionized Water	77.32	0.20	2.15
Methanol	22.66	0.34	1.06
Silica (Granular)	2.83	0.003	0.013

TABLE II

ELECTRICAL PROPERTIES OF PERFUSATE SOLUTION AS A FUNCTION OF DMSO CONCENTRATION AT 2450 MHz MEASURED USING WAVEGUIDE MEASUREMENT SYSTEM

DMSO Concentration	Dielectric Constant	Loss Tangent	Conductivity (mho/m)
Frozen Perfusate Solution:			
0%	3.18	0.01	0.03
5%	4.23	0.39	0.25
10%	5.73	0.25	1.97
15%	8.25	0.19	2.36
Thawed Perfusate Solution:			
0%	58.68	0.20	16.36
5%	56.97	0.20	15.29
10%	56.02	0.22	17.33

from sample to sample depending upon the sample surface texture. Various techniques for slicing tissue and for placing the tissue in the waveguide system were investigated in order to determine a solution to the problem of sensitivity to surface smoothness. The most accurate results were obtained when the kidney tissue was "diced" into rectangular slices of uniform thickness and then placed side by side in contact with each other. Measurement results of the electrical properties of kidney tissues at a frequency of 2450 MHz using these sample-preparation techniques are shown in Table III.

Although dicing of the tissue greatly improved the results of the waveguide electrical property measurements, other techniques for improving the repeatability of the measurements and for circumventing some of the problems associated with tissue sample preparations were examined. Because of the limitations associated with the waveguide technique and of the desirability for the capability to accurately measure small samples, a coaxial transmission line measurement system was investigated. In the coaxial system, small disk samples were placed between the end of the center conductor and the shorting plate at the end of the coaxial line, as indicated in Figure 3. The accuracy of this system was determined through the measurement of the properties of disk samples of known dielectric constants. The results of measurements on paraffin and on two stycast materials having reported dielectric constants of 4.0 and 15.0 known to within approximately 10 percent, respectively, are given in Table IV.

Although measurements of the electrical properties of kidney tissue have been previously reported [8,18], these measurements did not include data at microwave frequencies on either frozen kidneys or kidneys containing

TABLE III

ELECTRICAL PROPERTIES OF KIDNEY TISSUE AT 2450 MHz
MEASURED USING WAVEGUIDE MEASUREMENT SYSTEM

Sample	Dielectric Constant	Loss Tangent	Conductivity (mho/m)
Rabbit Kidney Cortex:			
Frozen	3.06	0.03	0.16
Thawed	70.79	0.26	2.70
Canine Kidney Cortex:			
Thawed	72.20	0.29	2.83

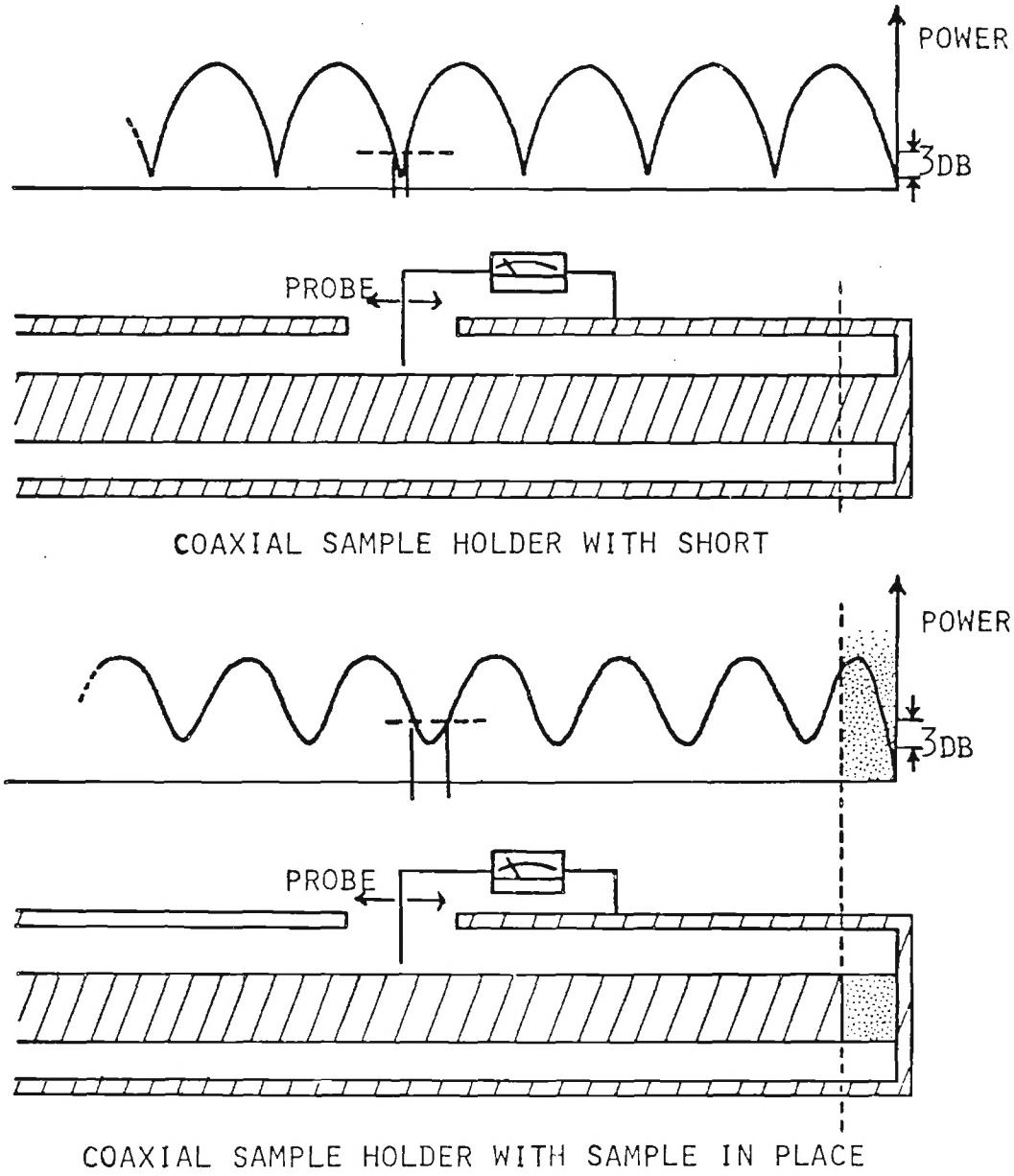


Figure 3. Coaxial measurement system.

TABLE IV

ELECTRICAL PROPERTIES OF SAMPLE STANDARDS AT 2450 MHz
MEASURED USING COAXIAL MEASUREMENT SYSTEM

Sample	Dielectric Constant*	Loss Tangent	Conductivity (mho/m)
Paraffin	2.58	0.08	0.03
K4	4.25	0.002	0.001
K15	16.21	0.02	0.03

* Paraffin has a dielectric constant of 2.5 $\pm$ 5%

* K4 has a dielectric constant of 4.0 $\pm$ 10%

* K15 has a dielectric constant of 15.0 $\pm$ 10%

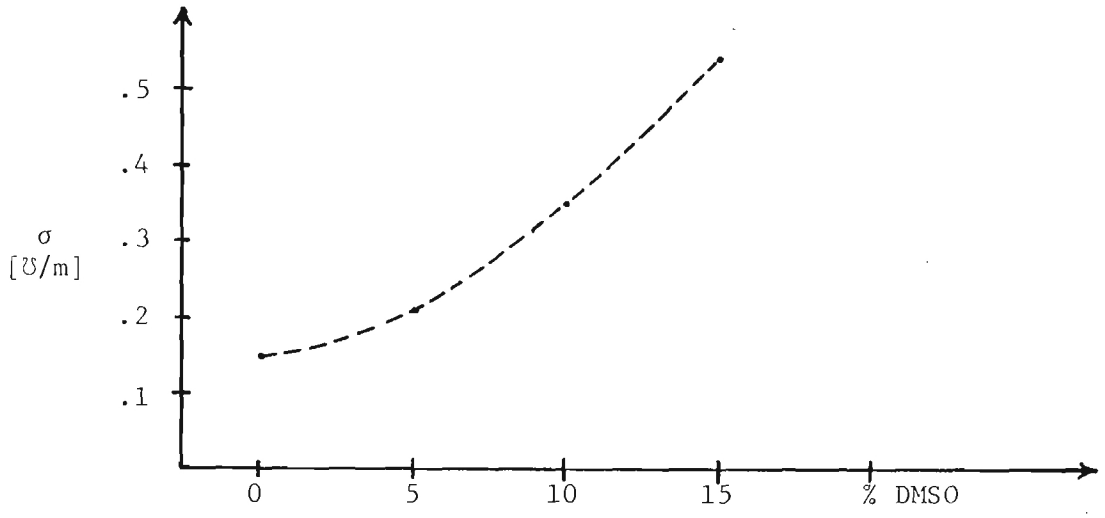
cryoprotectant drugs. Measurements of the relative dielectric constant and loss tangent for rabbit kidneys perfused with a Mg++-K+ rich perfusate solution with 2% HES containing four concentrations (0,5,10, and 15%) of DMSO were performed for both frozen and thawed tissue at a frequency of 2450 MHz. These results are summarized in Figures 4 and 5. This knowledge of the relative dielectric constant and loss tangent of the tissue allows the determination of the penetration depth (distance in which the incident electromagnetic fields have been reduced to 0.368 of its incident value) of the energy incident upon the kidney. As shown in Table V, the penetration depth changes significantly with temperature.

Measurements were also performed to assess the repeatability of the coaxial measurement system. Table VI shows the results of measurements performed for four samples of rabbit kidney cortex taken from a single rabbit kidney. These results indicate very good repeatability of the coaxial system for separate and independent tissue samples. The electrical properties of kidney cortex shown in Figure 6 were also measured as a function of frequency over the range from 2.3 GHz to 3.0 GHz using the coaxial measurement system. These results indicate that for tissue sample measurements in the microwave frequency range between 2 and 3 GHz, especially where the sample size is limited, the coaxial measurement technique employing small disk samples provides useful results having both reasonable accuracy and repeatability.

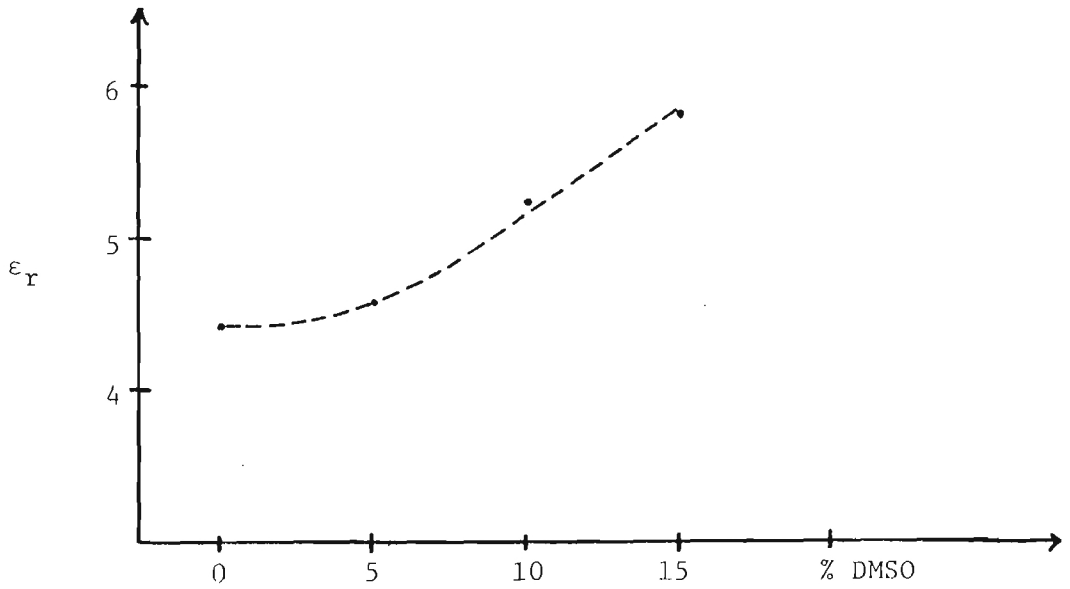
B. Analytical Modelling of Internal Field Configuration

The mechanisms by which electromagnetic fields penetrate biological tissues were investigated analytically, and initial models for predicting internal electromagnetic field configurations have been developed. Until

FROZEN SAMPLES



KIDNEY TISSUE CONDUCTIVITY(σ) vs. DMSO CONCENTRATION



KIDNEY TISSUE PERMITTIVITY(ε_r) vs. DMSO CONCENTRATION

Figure 4. Electrical properties of frozen rabbit kidney tissue for four levels of DMSO concentration.

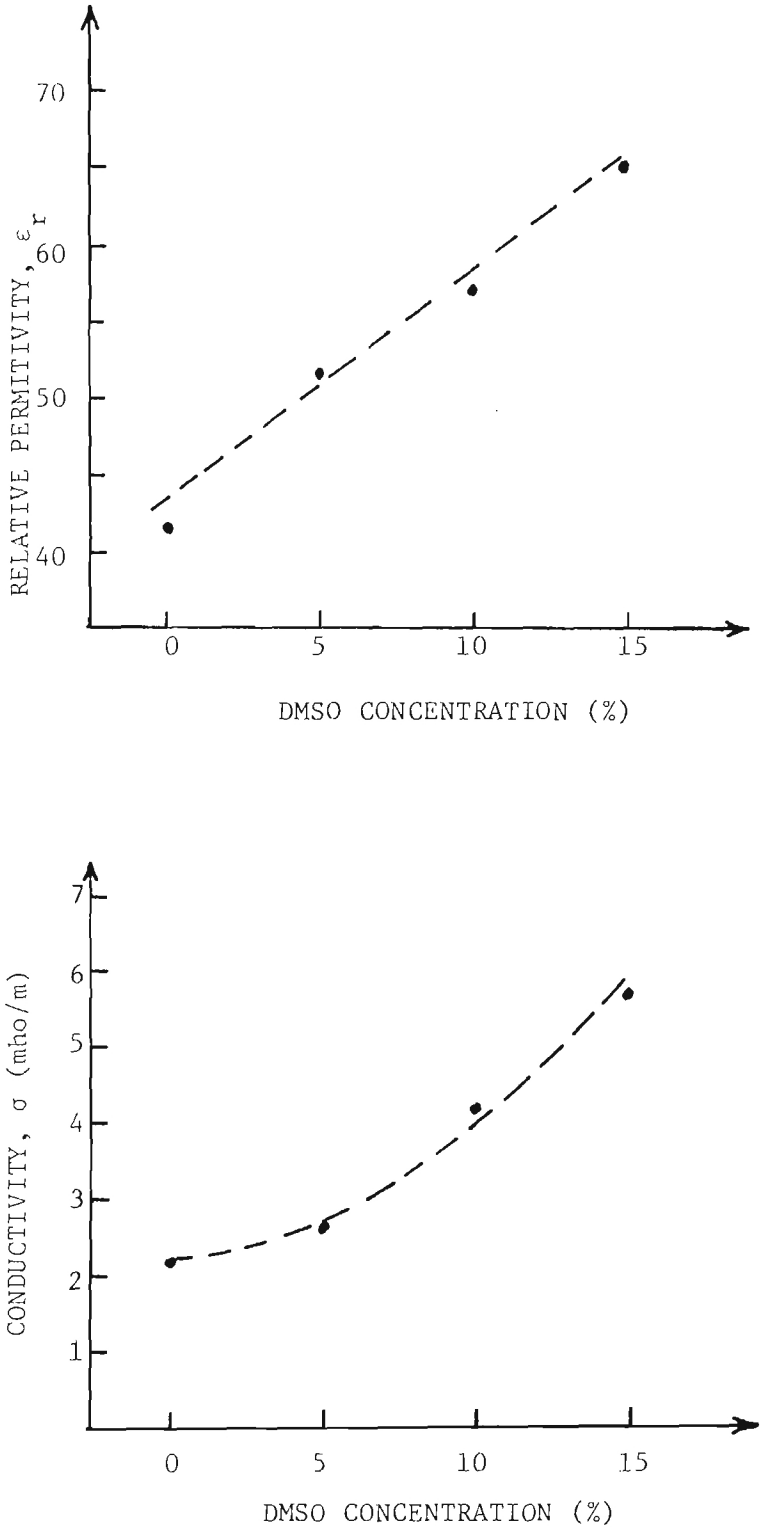


Figure 5. Electrical properties of thawed kidney tissue at 2450 MHz for four levels of DMSO concentration.

TABLE V

PENETRATION DEPTH OF 2450 MHz RADIATION IN KIDNEY TISSUE AT VARIOUS TEMPERATURES

Temperature (°C)	Dielectric Constant	Penetration Depth (cm)
25	68	1.59
10	63	1.65
- 1	57	1.32
-20	4.3	5.64

TABLE VI

ELECTRICAL PROPERTIES OF FOUR CORTEX TISSUE SAMPLES CUT FROM THE SAME RABBIT KIDNEY AT 2450 MHz MEASURED USING COAXIAL MEASUREMENT SYSTEM

Sample	Dielectric Constant	Loss Tangent	Conductivity (mho/m)
I	73.29	0.48	4.81
II	72.56	0.45	4.41
III	72.69	0.65	6.39
IV	72.96	0.52	5.14
Average	72.88 $\pm$ 0.32	0.52 $\pm$ 0.09	5.19 $\pm$ 0.86

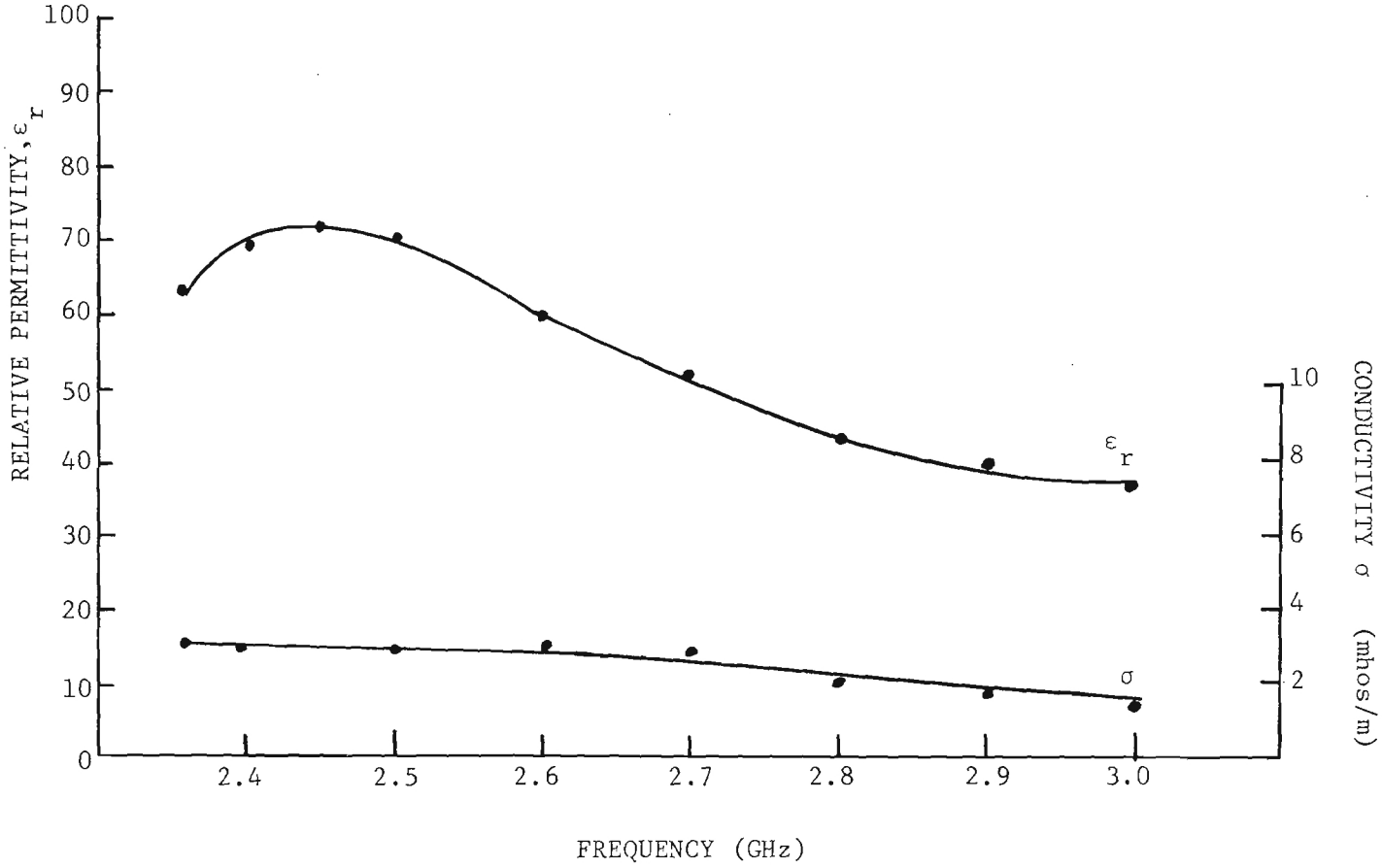


Figure 6. Electrical properties of thawed kidney cortex as a function of frequency.

recently, analytical methods to determine the field distribution within samples of biological material have been primarily limited to the classical Separation of Variables methods, and thus have been restricted to a sphere or a circular cylinder [19-21]. In order to obtain more realistic geometrical models with varied and arbitrary contours, the use of techniques which lend themselves readily to numerical solution is particularly advantageous. The Method of Moments [22], which has been extensively employed in other types of electromagnetic problems, is such a technique. The advantage of applying Moment Method techniques is the ability to determine the EM field distribution within whole organs to predict the locations of potential non-uniform heating in realistic geometrical models of varied and arbitrary contours, such as rabbit kidneys.

Currently, a computer program based on the Method of Moments is being used to predict the field intensity within sample models represented by circular and elliptical cylinders when a plane electromagnetic wave is incident on the sample [23]. For example, when rabbit kidneys are represented by specifying the conductivity, relative dielectric constant, and permeability of the tissue, employing the elliptical cylinder program to predict the internal field configurations in frozen and thawed rabbit kidneys yields the results shown in Figures 7 and 8, respectively. Similar results are shown for canine kidneys in Figures 9 and 10. Note that the predicted field intensity within the frozen tissue is significantly higher and more uniform than in the thawed tissue. This fact was confirmed through laboratory tests in which the reflected power increased significantly as the tissue changed phase state, which indicates that less energy penetrated the kidney [10].

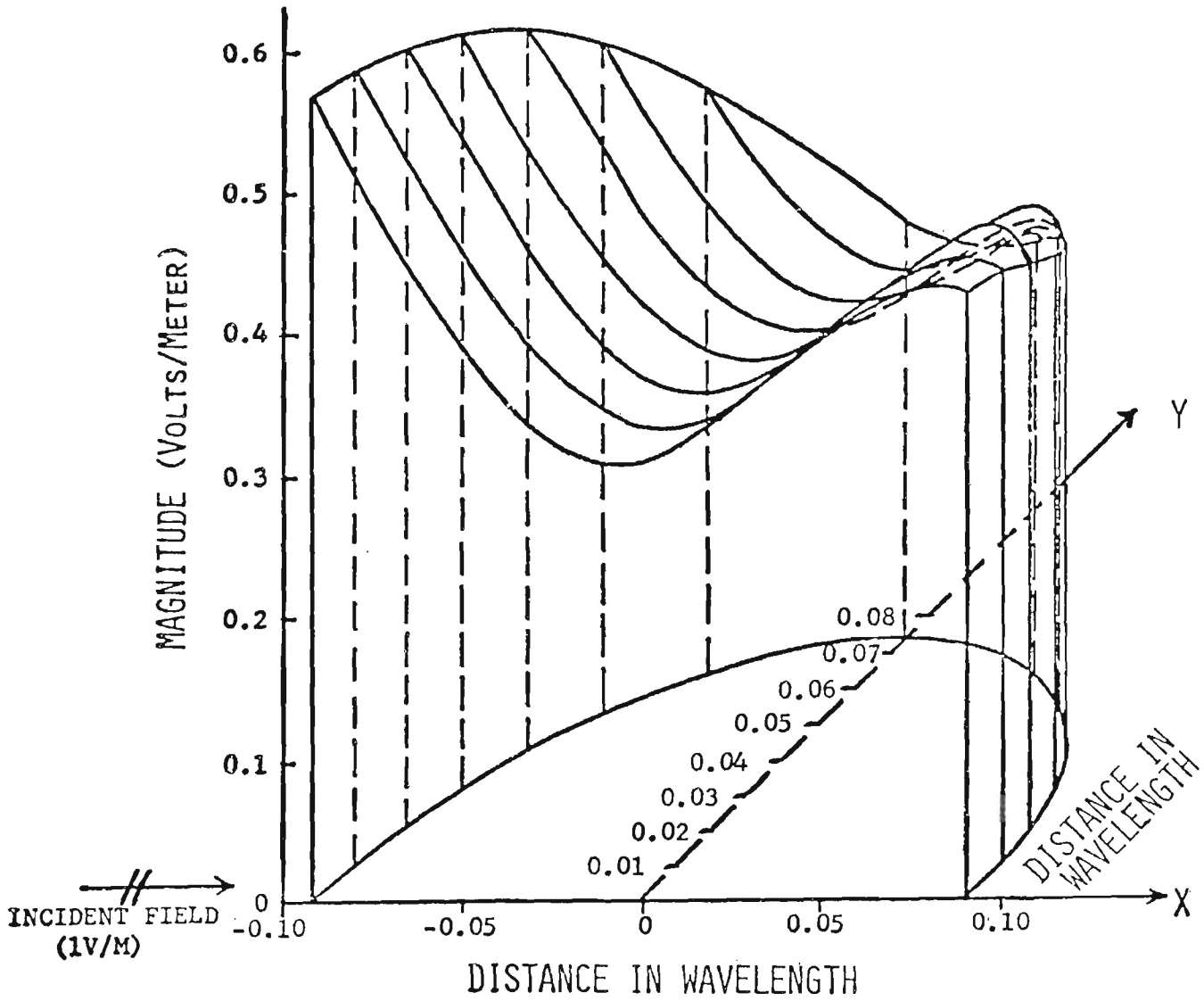


Figure 7. Field strength in frozen rabbit kidney at 2450 MHz for $\varepsilon = 3.7$ and $\sigma = 0.04$.

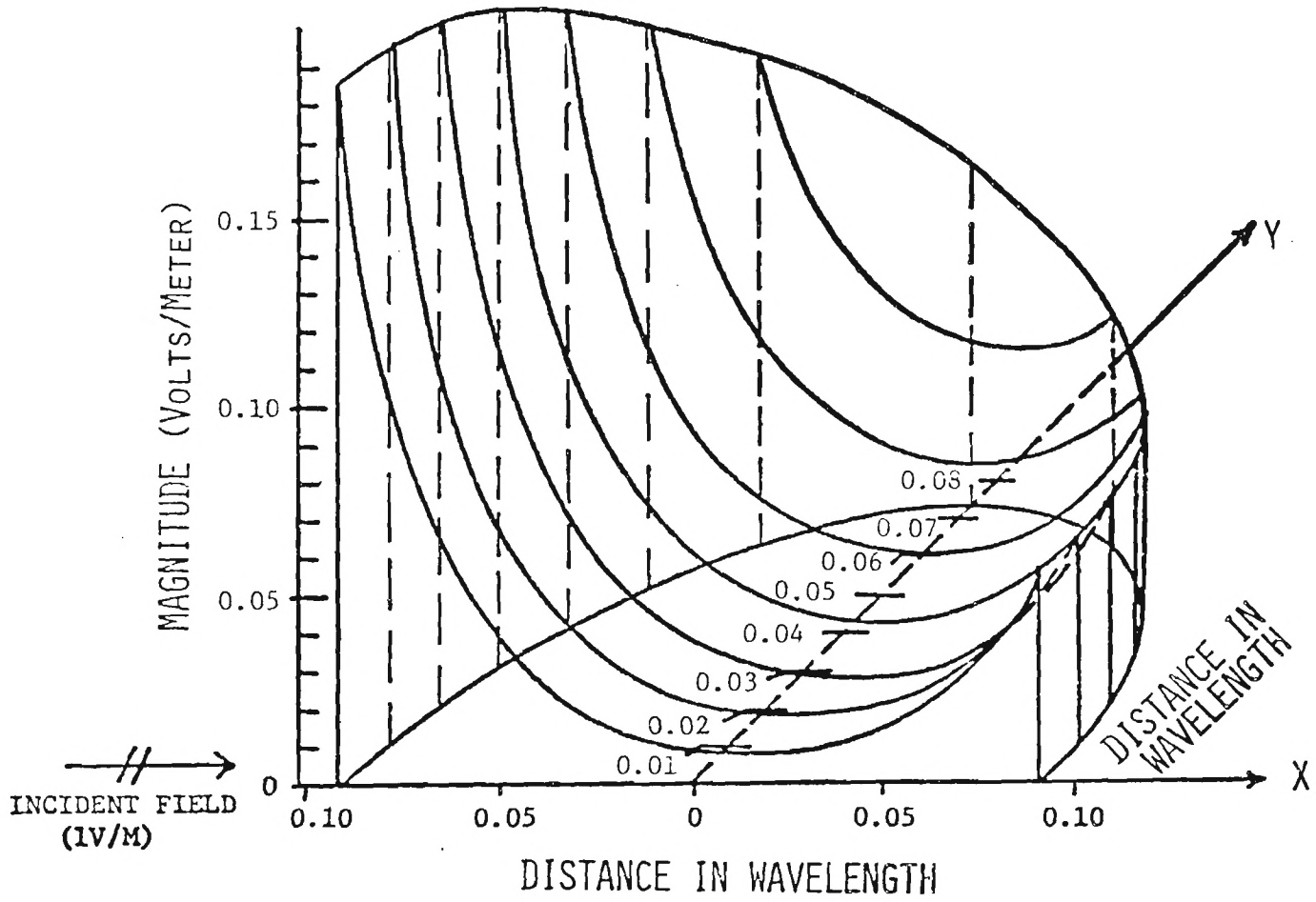


Figure 8. Field strength in thawed rabbit kidney at 2450 MHz for $\varepsilon = 51$ and $\sigma = 3.0$.

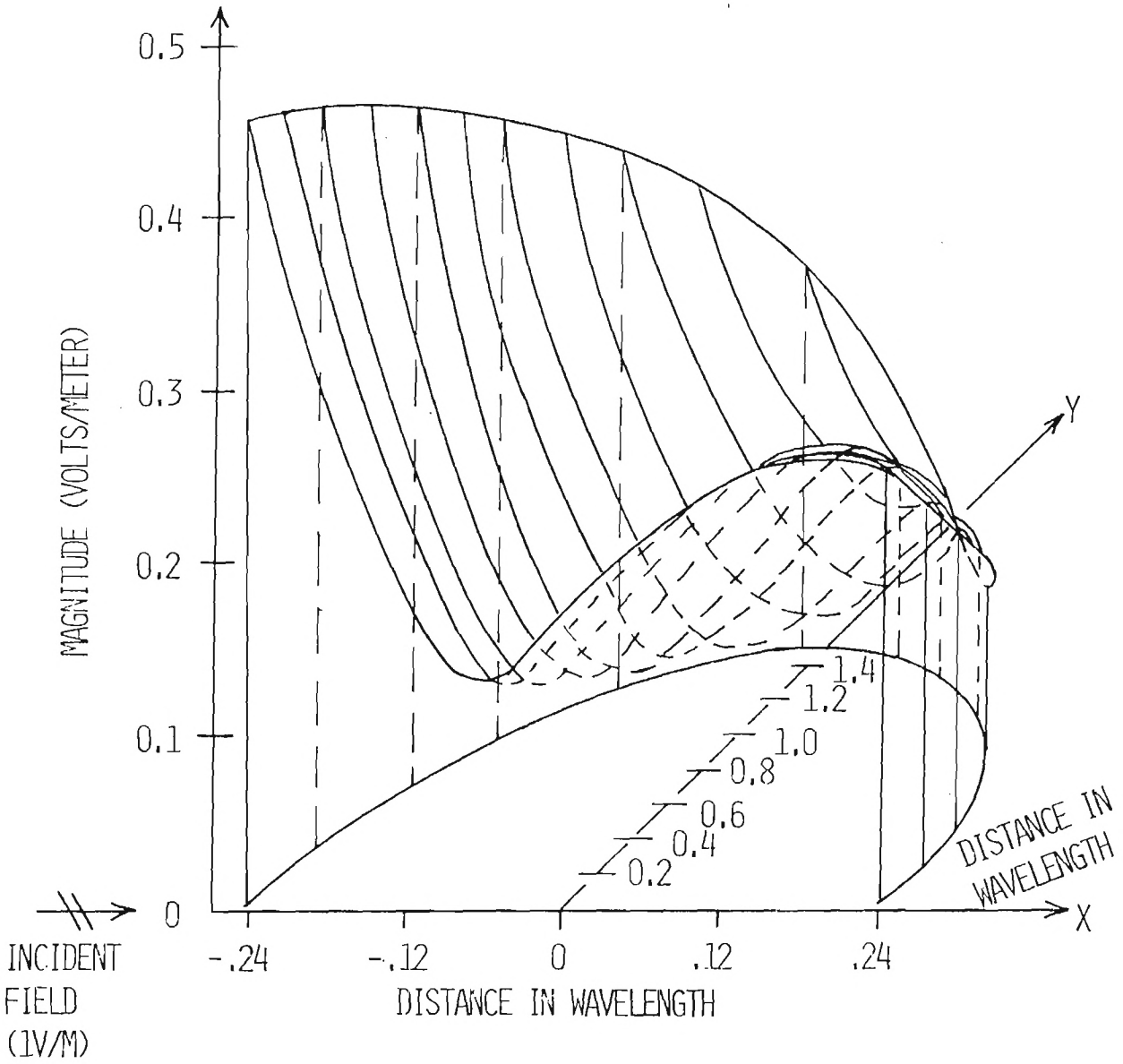


Figure 9. Field strength in a frozen canine kidney at 2450 MHz for $\varepsilon = 3.9$ and $\sigma = 0.1$.

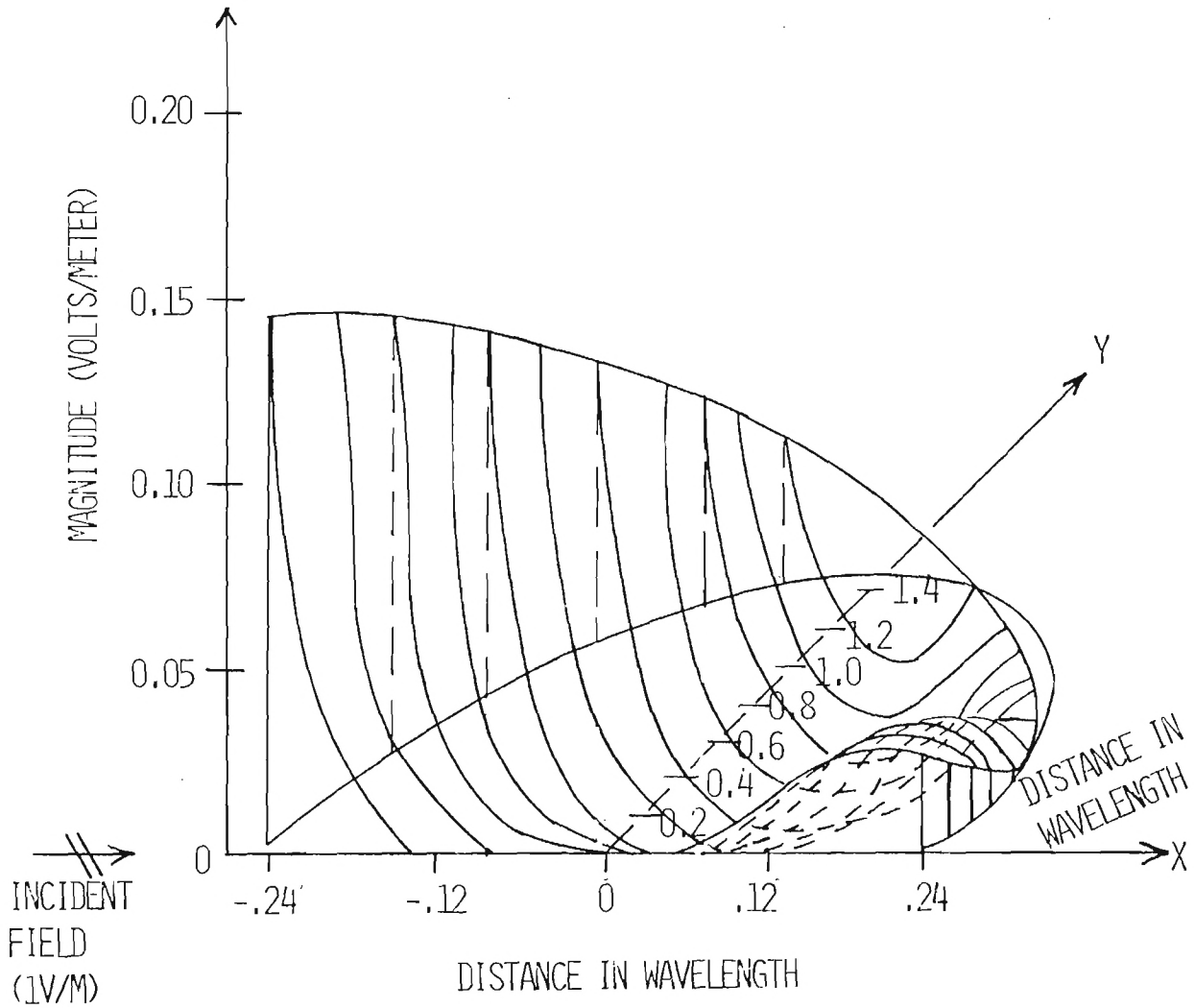


Figure 10. Field strength in a thawed canine kidney at 2450 MHz for $\varepsilon = 60$ and $\sigma = 3.0$.

Determination of the absorbed energy in this manner, which utilizes the electrical properties of the kidney and integral solutions of the internal fields which predict resonance effects, yields a solution which is as accurate as the modelling geometry permits.

In deriving an optimum thawing technique, various tradeoffs among the electrical parameters are necessary. In addition to the dielectric constant and loss tangent, these parameters include the radiation frequency, the illuminator design, the type of modulation, and the incident power level. Therefore, based on the electrical properties of the frozen and thawed kidneys for specified levels of cryoprotectant and for specified sizes of organs, it is necessary to select the proper combinations of the electrical parameters to obtain the required thawing rate and uniformity.

C. Electromagnetic-Thawing System Designs

An examination of penetration depth of electromagnetic radiation into frozen rabbit and canine kidneys, as a function of frequency, immediately indicates that uniform thawing of rabbit kidneys could be achieved by 2450 MHz radiation alone, but not for canine kidneys. Therefore, both single-frequency and multifrequency systems were designed. Moreover, the technical requirements for the effective thawing of cryopreserved kidneys also include the following:

1. selection of a proper microwave source to provide a continuously variable and controllable output-power level,
2. a tuning device to provide effective electrical matching of the microwave source impedance to that of the illuminator,
3. instrumentation to provide for continuous monitoring of the forward and reflected power levels,

4. an illuminator design based on the compatibility of the illuminator's field distribution with the electrical properties of the tissue in order to obtain uniform internal field configurations throughout the region to be thawed, and
5. temperature information for controlling the power level of the source and the endpoint temperature of the thawed kidney.

Since commercial equipment with the desirable variable power capabilities were not available, existing power supplies for the single-frequency and multi-frequency systems were adapted for use with their associated power sources. The block diagrams of Figures 11 and 12 illustrate the major components of the two arrangements of the electromagnetic thawing systems. These instrumentation arrangements shown in these figures permit both the transmitted and reflected power to the illuminator to be continuously monitored. The tuners in the transmission lines permit matching of the source impedance to that of the load in the frozen state in order to minimize the power reflected back to the source from the load.

1. Description of Single-Frequency Thawing System

The high-power microwave source employed in the 2450-MHz thawing experiments is capable of operation at power levels up to 1500 watts for periods of approximately 10 minutes and at lower power levels for considerably longer periods of time. The adjustable power divider shown in Figure 11 allows the power supplied to the illuminator to be varied from less than a watt to the maximum output level of the magnetron power tube. When power is diverted from the applicator, the excess diverted power is directed into high power terminations. The two directional couplers located between the power divider and the illuminator are used to sample known amounts of power.

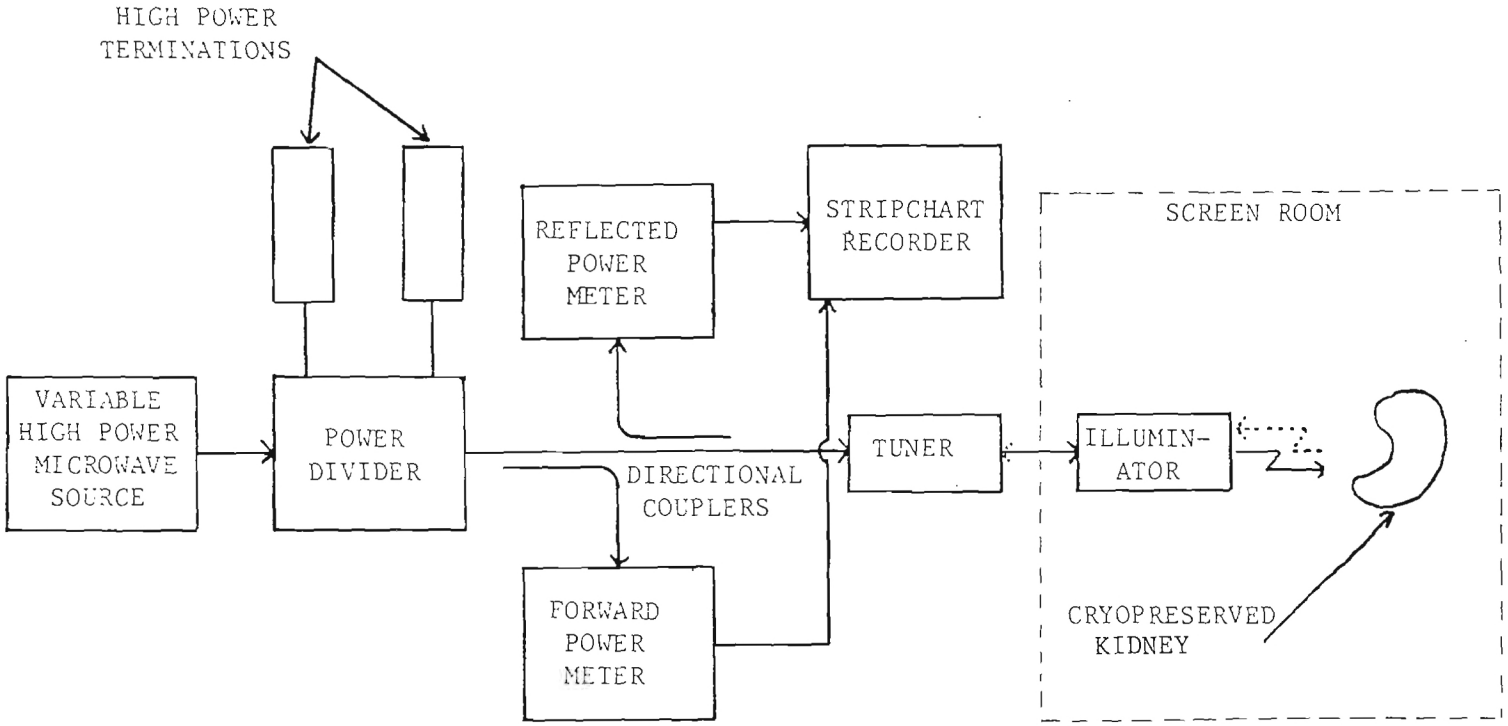


Figure 11. Block diagram of instrumentation system showing components of the source and microwave monitoring equipment.

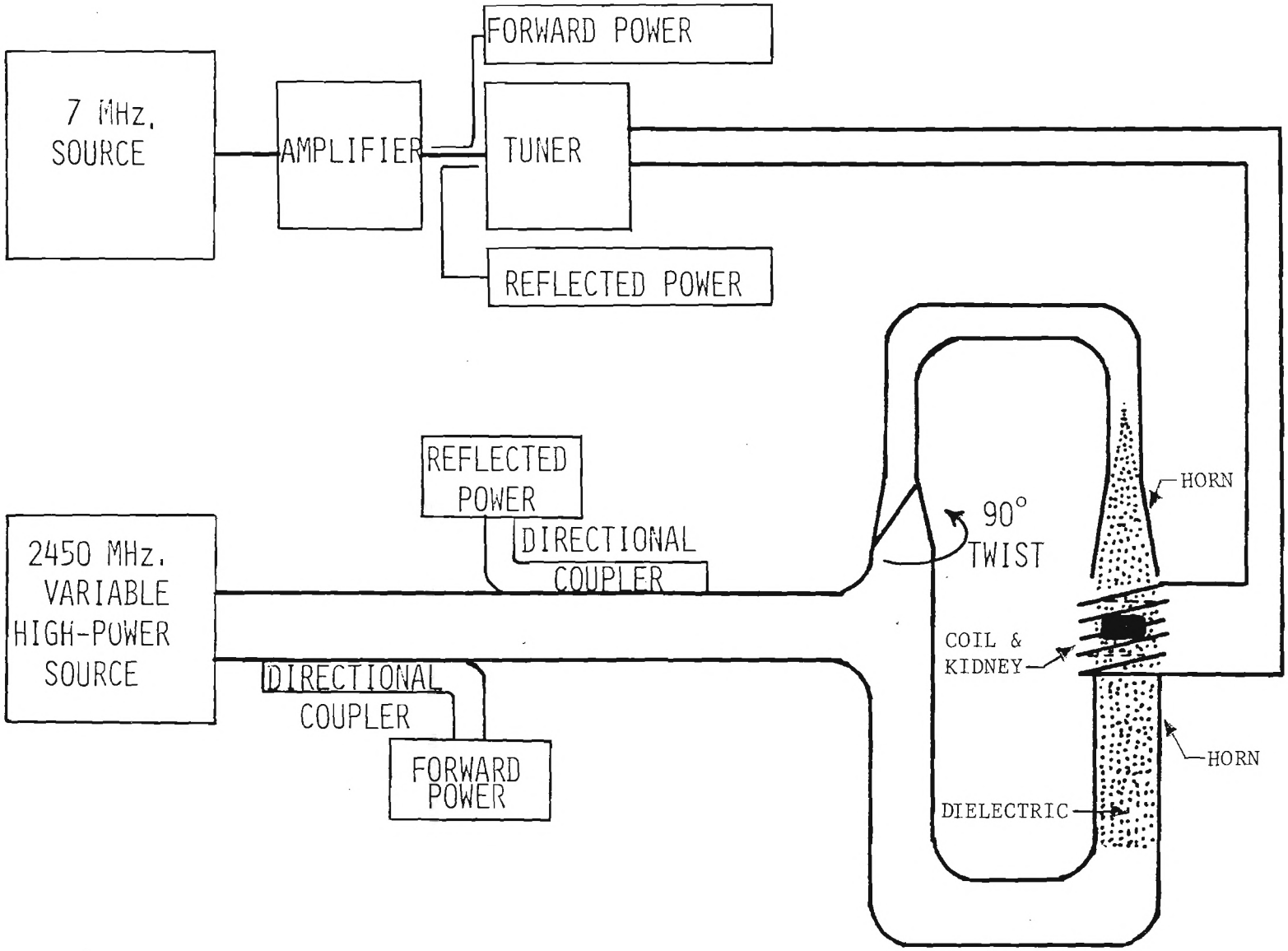


Figure 12. Block diagram of multifrequency thawing system showing sources, illumination system, and power monitoring equipment.

One coupler samples a known fraction of the power fed to the illuminator from which the total power reaching the illuminator is accurately determined. The second directional coupler samples the power reflected back from the load.

A dielectric-loaded flared horn illuminator was designed to dielectrically match the kidney tissue in the frozen state. Silicon dioxide (SiO_2) in granular form, which has a dielectric constant of slightly less than three and is very close to that of frozen kidney tissue at a frequency of 2450 MHz, was used in the innnermost part of the horn, and titanium dioxide (TiO_2), which has a dielectric constant of approximately nine in powdered form, was used to load the edges of the horn illuminator. This dielectric loading configuration, which is shown in Figure 13, resulted in an aperture illumination function which is more uniform than the aperture illumination function for pure SiO_2 dielectric loading. An examination of the previous analytical results of the internal field configurations indicates that a higher and more nearly uniform field intensity is observed in the frozen rabbit kidney than in the thawed kidney, which indicates the match between the dielectric loading materials and the electrical properties of the kidney is better for the frozen state than for the thawed state. The illuminator was mounted in the screen room and was oriented to face the microwave absorbing material in order to absorb that portion of the microwave energy which propagated through the frozen kidneys. This arrangement prevents the occurence of standing waves between the shielded room and the illuminator; consequently, the measured reflected power is entirely attributable to the presence of the kidney.

2. Description of Multifrequency Thawing System

The multifrequency system shown in Figure 12 was developed for the simultaneous use of electromagnetic radiation at two widely separated frequencies to produce controllable field configurations for thawing frozen organs. For frequencies in the HF region, uniform fields within the frozen kidney can be achieved; however, the tissue becomes almost transparent and little heating occurs. It was found that the center of the frozen kidney could be heated with HF radiation if tiny stainless steel spheres were inserted through the ureter cannula into the medulla of the kidney before it is frozen. Because the spheres heat rapidly in the HF field, induction heating occurs from the interior of the kidney outward. Microwave radiation at 2450 MHz can produce heating that is the greatest at the surface of the kidney and decreases with depth of penetration. Thus, by carefully monitoring and controlling power level and heating time of both the HF and microwave sources, one can achieve rapid and uniform thawing of a frozen kidney.

Two horn antennas connected by a dielectric waveguide whose dielectric material is a silicon dioxide (SiO_2) powder comprised the 2450-MHz illuminator. An induction coil encircling the dielectric waveguide is the 7-MHz illuminator for magnetic heating of small stainless steel spheres that are inserted into the kidney before freezing. The two horn-antennas are oriented such that their polarizations are orthogonal to minimize antenna coupling and reflected energy to the source. The frozen organ can be imbedded in the dielectric powder to minimize discontinuities in the medium, and a tuner is provided with the 7-MHz source to maximize coupling into the frozen organ.

As was the case for the single-frequency thawing system, means are also provided for monitoring the forward and reflected power levels on the

transmission lines from both sources in the multifrequency system. In either thawing system, a significant increase in reflected power occurs when the organ goes from the frozen to the thawed state. This phenomenon can be used as an indicator in the power control. The output power from both sources can be accurately controlled with maximum outputs of approximately 1500 watts for the 2450-MHz source and 600 watts for the 7-MHz source.

D. Electromagnetic Thawing Results

Temperature measurements of kidneys during thawing are necessary to provide real-time data on the heating distribution and rate. These data would be useful for optimization of thawing system design as well as functioning as an input for system power control. However, conventional methods of temperature measurement do not completely satisfy the requirements of an acceptable measurement technique for use with EM thawing systems.

Sensors which are adequate for measurement of the temperature rise of biological tissue in the presence of electromagnetic fields are a major problem [24-26], and investigators have used various techniques [27,28] depending on the type of experiments being performed. Evaluations of various types of thermocouples and thermistors for use in measuring temperature rise in the presence of an electromagnetic field have been made. These evaluations indicate that thermocouples are generally unusable because they induce focal heating in the tissue adjacent to the device. Focal heating results from electromagnetic heating of the metallic sensor, thus resulting in an erroneous temperature reading. On the other hand, a thermistor bead, which is a resistive material rather than a metallic wire junction, tends to perturb the fields within the tissue less than a thermocouple. However, the wire leads attached to the thermistor bead are not

transparent to electromagnetic energy because of their relatively high conductivity (even nichrome has a conductivity of 10^6 mhos/meter as compared to a typical value of 1 mho/meter for tissue), but this problem can be minimized by placing the leads perpendicular to the electric field, thereby reducing heating of the leads and minimizing the field perturbations. The induced current in the leads as a function of orientation with respect to the electric field is indicated in Figure 14. Further, high-resistance thermistors with carbon impregnated teflon leads whose conductivities approach that of tissue have been used in an electromagnetic field, and temperature measurements have been recorded with little or no measurement error [28].

Although certain types of temperature measurement probes are transparent to electromagnetic radiation [28-30], a non-invasive temperature sensor would be the best alternative, from a clinical standpoint, for measuring the temperature of tissue during thawing. A very encouraging non-invasive temperature monitoring technique which employs the power reflected from the load (kidney) during thawing as an indicator of phase state was investigated. This non-invasive technique utilizes the fact that the electrical properties of the tissue changes as the frozen organ thaws; consequently, the power reflected back to the source increases significantly. The reflected power increases because the dielectric constant and conductivity of the tissue changes as the tissue changes from the frozen to the thawed state, and therefore the penetration depth of the microwave energy and the reflection coefficient of the tissue also change. Curves of both reflected power and temperature as a function of the time that a frozen kidney is

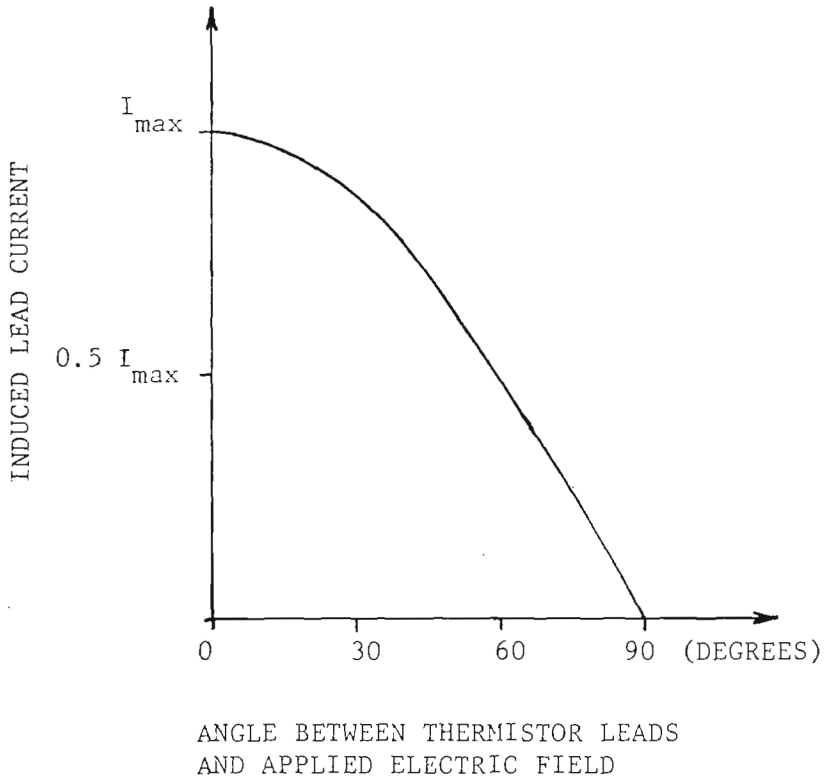


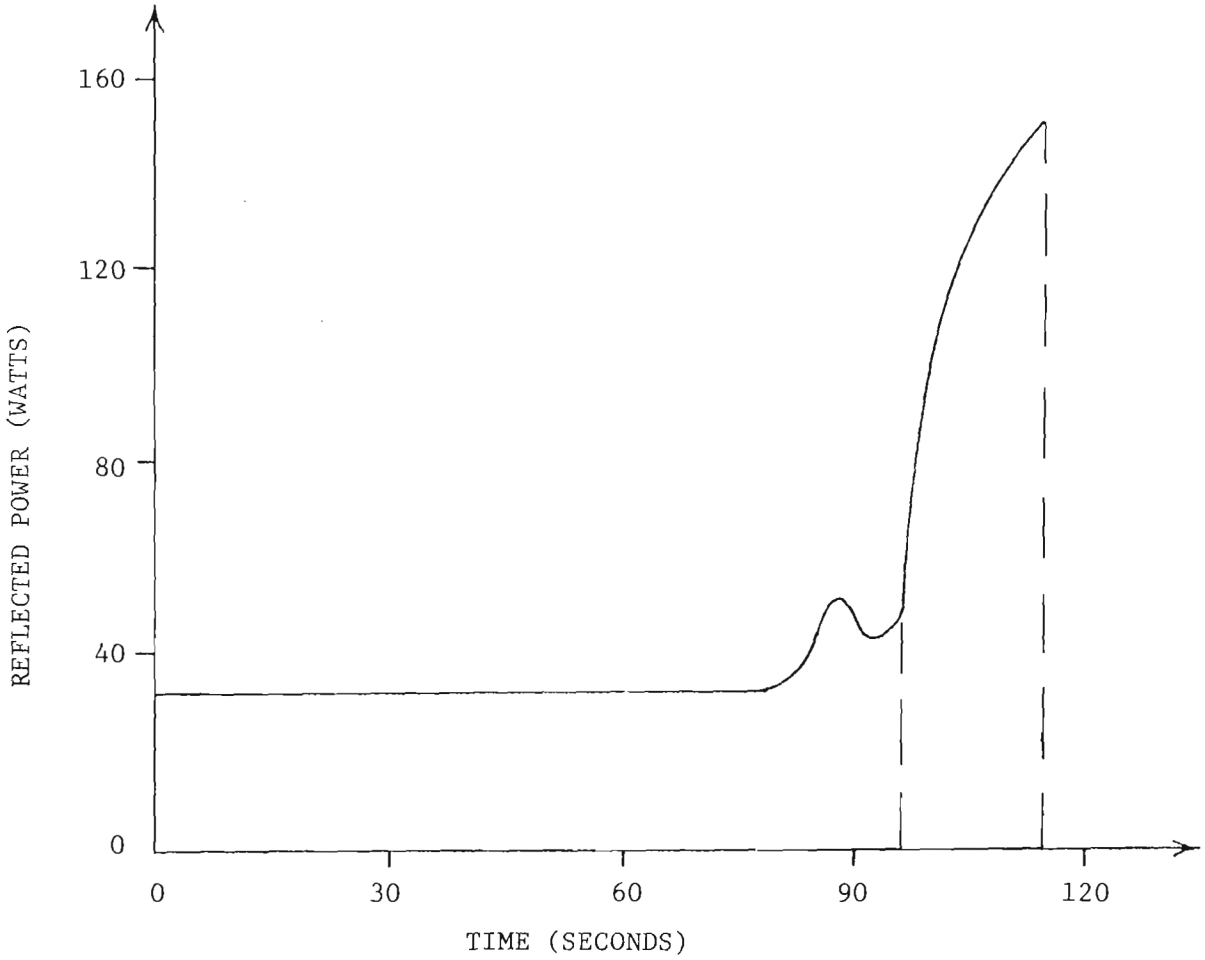
Figure 14. Coupling of electric field as a function of angular placement of thermistor.

illuminated with electromagnetic energy is shown in Figure 15. The shapes of these curves are typical for both rabbit and canine kidney investigations. The reflected power begins to increase a short time before the change of phase, which occurs at about 80 seconds in the figure. A steep increase in reflected power is observed after the change of phase.

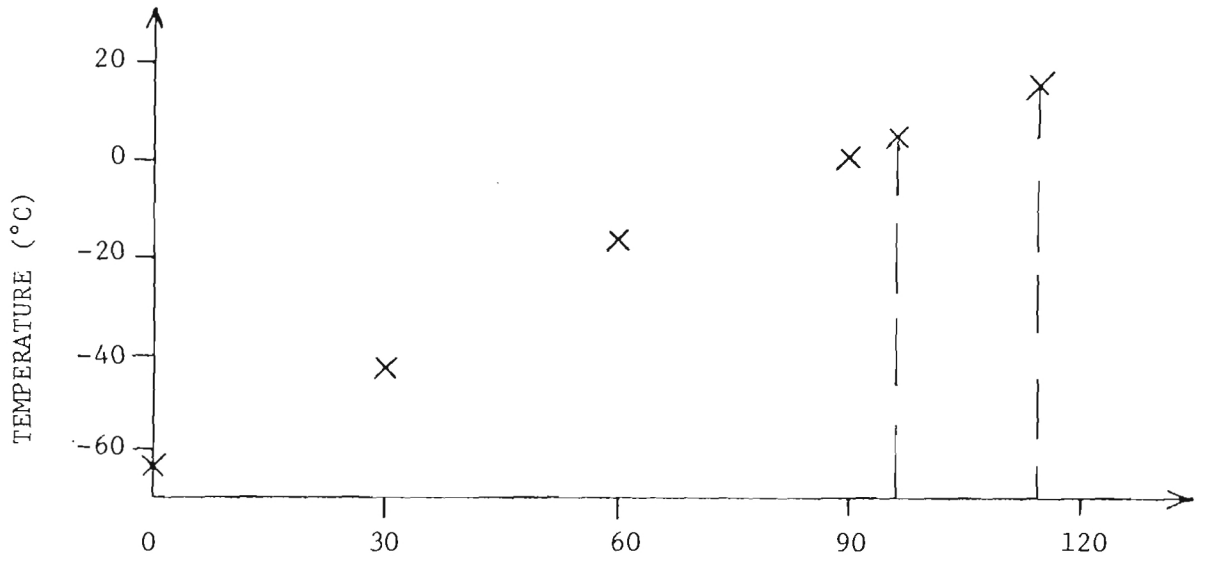
1. Rabbit Kidney Results

Both the measurement of reflected power and of temperature using small-bead thermistors were recorded during various thawing investigations. The thawing results indicated in Figure 16 for a rabbit kidney irradiated with a dielectrically-loaded flared horn illuminator at a frequency of 2450 MHz illustrate the thawing rate and uniformity for a kidney thawed from -80°C. An independent check of the temperature at four points on the surface of the kidney immediately after thawing indicated a maximum temperature differential of 8°C over the surface. If an automated power level/temperature control system were implemented, the temperature differential over the surface could be further reduced, and the final temperature of the thawed kidney could be precisely controlled.

During this research program, the single-frequency (2450 MHz) electromagnetic illumination system described above was utilized in various thawing investigations involving rabbit kidneys. A total of 82 frozen rabbit kidneys (provided by Dr. A.M. Karow of the Medical College of Georgia) were thawed, and their viability assessed via post-thaw perfusion and histological examination of kidney slices. A statistical analysis of kidney slices was performed for various levels of the cryoprotectant DMSO in the perfusate solution. Further, the weight gain of the organ during perfusion was



(a) Reflected Power



(b) Measured Temperature

Figure 15. Plot of (a) reflected power and (b) measured temperature as a function of time.

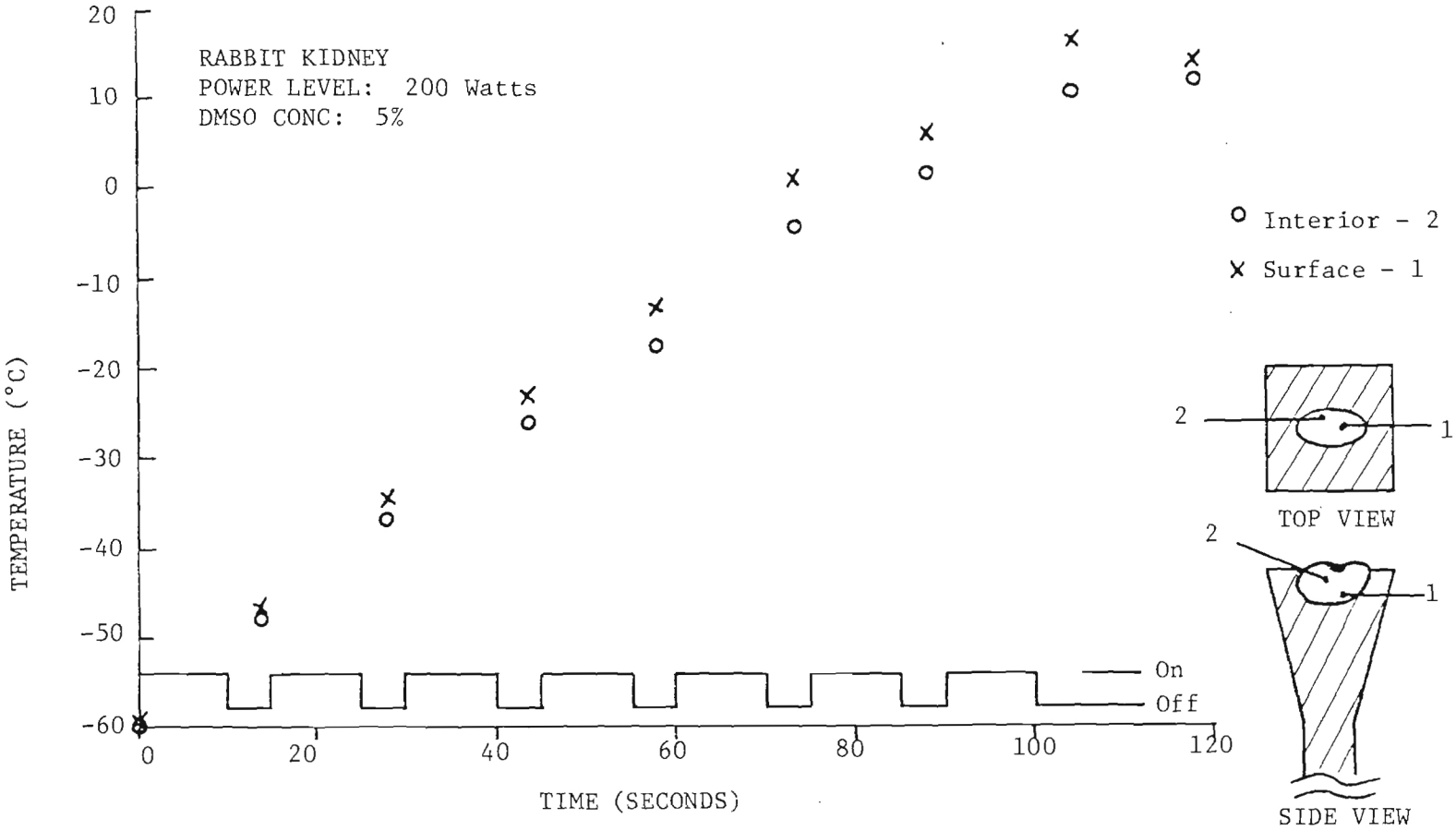


Figure 16. Temperature measured at the surface and interior of rabbit kidneys as a function of time using pulsed 2450-MHz radiation.

correlated (1) with the uniformity evaluation of the kidney slices performed immediately following EM thawing and perfusion and (2) with the concentration of DMSO in the perfusate. Figures 17 and 18 show the relation of the macroscopic kidney slice evaluation and histological examination, respectively, as a function of DMSO concentration. Note the improvement in slice evaluation and histology with the presence of a cryoprotective drug. The additional data collected during both the pre-freeze and post-thaw perfusions were analyzed by Dr. A.M. Karow and his associates at the Medical College of Georgia. These encouraging results of improved post-thaw viability of rabbit kidneys underscore the great potential progess possible through implementation of electromagnetic thawing techniques.

2. Canine Kidney Results

The successful electromagnetic thawing of kidneys is a more difficult task for canine kidneys than for rabbit kidneys. Canine kidneys are significantly larger, and therefore, the 2450-MHz radiation of the single-frequency thawing system used to uniformly thaw rabbit kidneys does not penetrate completely through the large canine kidneys. Therefore, the multifrequency system described above was utilized to thaw frozen canine kidneys weighing approximately 65 grams. Prior to freezing, small bead thermistors were implanted in the kidney at locations indicated in Figure 19. The kidney was then imbedded into the dielectric powder within the medium connecting the two 2450-MHz illuminators.

It was found that more uniform thawing would result when the power level from both sources was modulated according to the pattern shown in Figure 19. Short periods when no power was applied helped to establish a thermal equilibrium within the organ, and less than maximum power was used

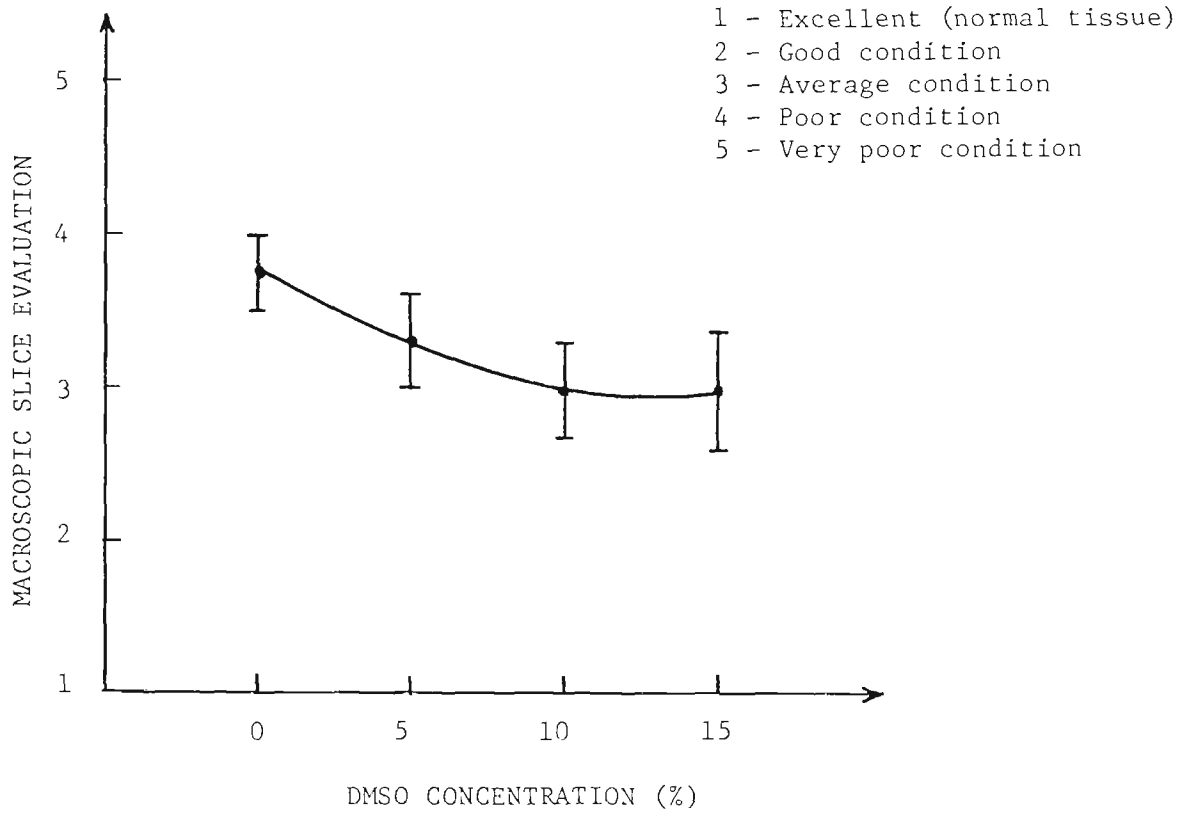


Figure 17. Results of macroscopic examination of kidney slices following EM thawing and perfusion for four levels of DMSO concentration in perfusate solution.

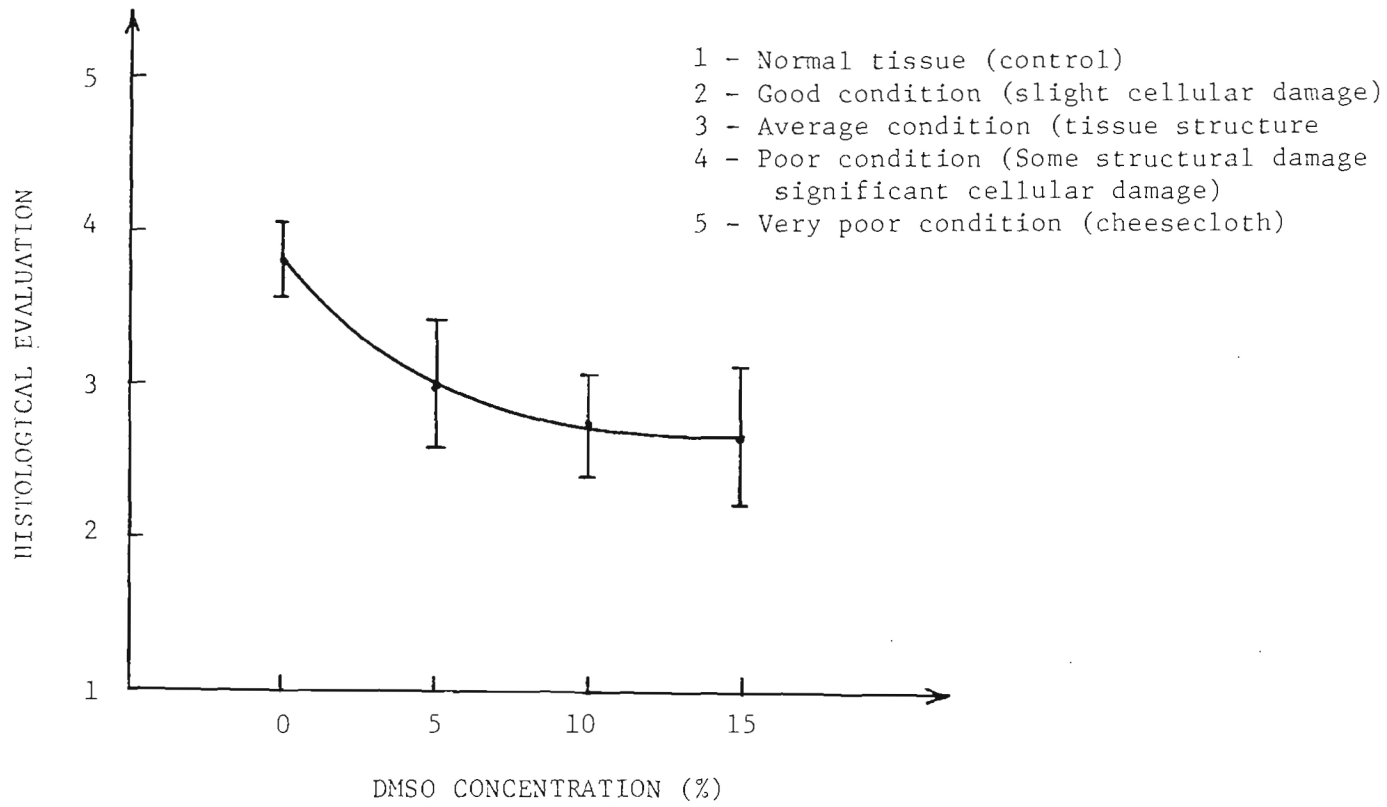


Figure 18. Results of histological examination of kidney slices for four levels of DMSO concentration in perfusate solution.

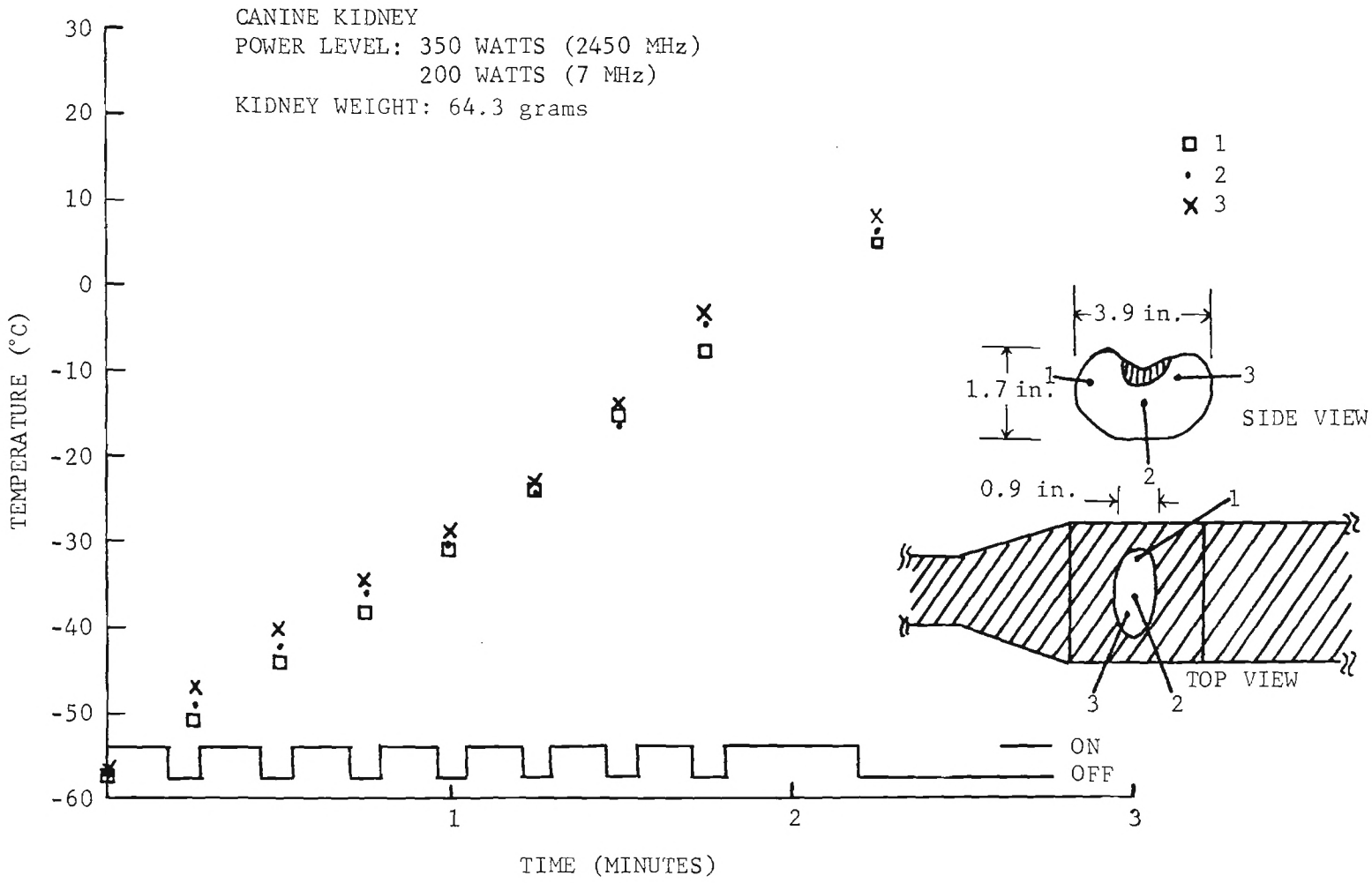


Figure 19. Temperature measured at three points in canine kidney as a function of time using combined 2450-MHz and 7-MHz pulsed radiation with recoverable doping material.

for both sources to permit a more controlled experiment. Higher powers will be used in future experiments when automatic power control of each source is implemented with the reflected power as an input parameter.

The kidney is heated from an initial temperature at approximately -60°C and thawed in about 2 minutes. Uniform heating and thawing is maintained as indicated by a comparison of the temperature profiles from the 3 implanted sensors. As the kidney temperature approaches the point where the change from the frozen to thawed state occurs, the applied radiation is kept on until the phase transition is complete. This longer duration of applied power is indicated by the longer pulse at the 2-minute time in Figure 19. This procedure aids in preventing recrystallization (the formation of large ice crystals at the expense of smaller crystals) that occurs when the kidney is warmed too slowly. If the smaller crystals recrystallize to form large crystals, cellular membrane structures are often destroyed. Applying radiation continuously during the phase transition helps prevent recrystallization by increasing the warming rate. However, this timing is critical because application of radiation even for a few seconds too long after the phase transition is complete will result in tissue burning due to the increased conductivity in the thawed state.

If only 2450-MHz radiation had been applied, the surface would have been heated and thawed in less than half the time of the center of the kidney as the reults of earlier experiments shown in Figure 20 indicate [31]. If only the 7-MHz source were used with the stainless steel spheres inserted nto the medulla of the kidney, the center would be thawed approximately rice as fast as the surface, as shown in Figure 21. Thus, it is only rough careful control of both power sources that uniform thawing can be ieved.

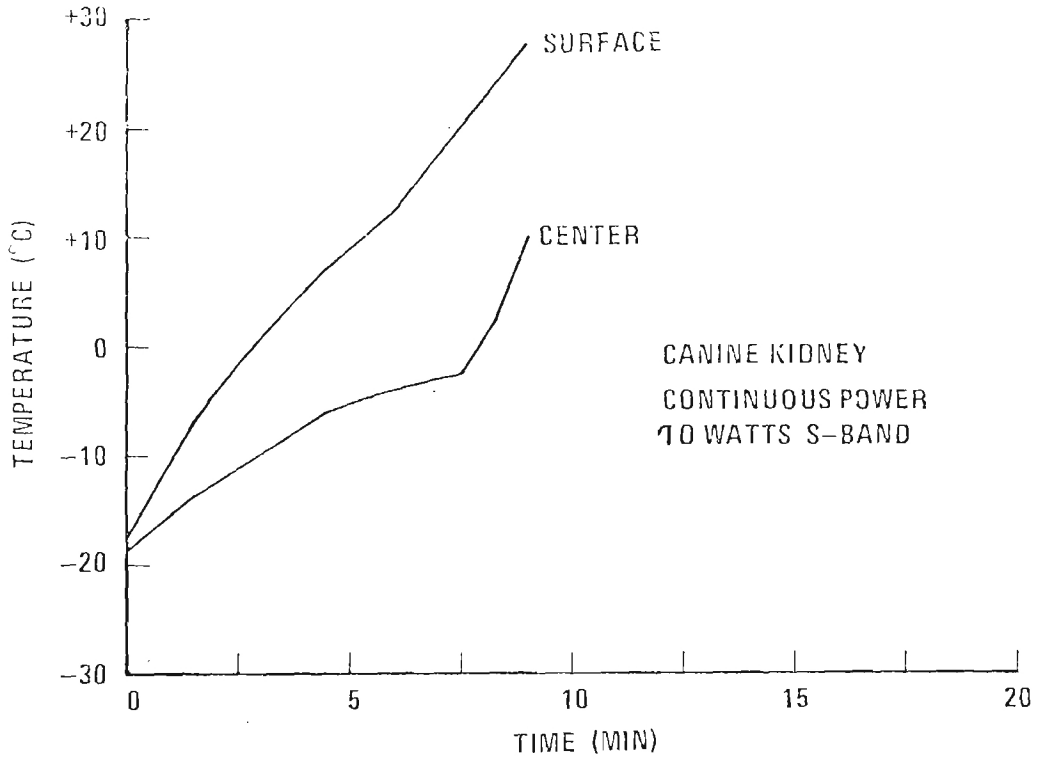


Figure 20. Temperature of the surface and center of frozen canine kidney versus time using continuous microwave radiation.

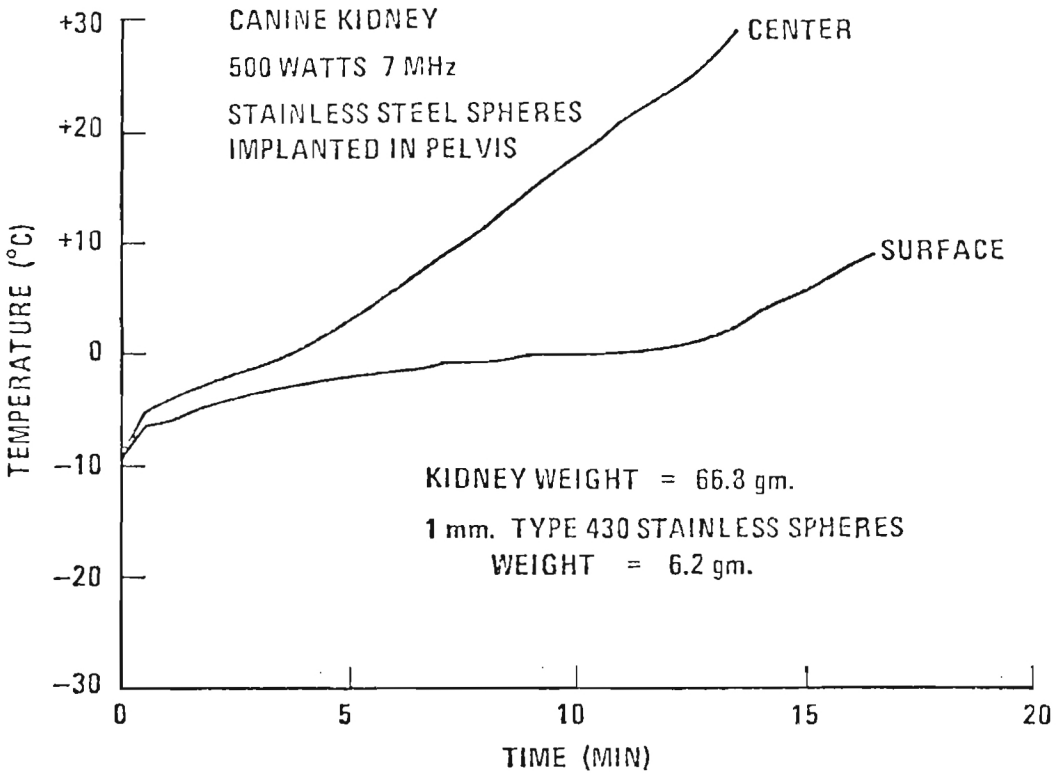


Figure 21. Temperature of the surface and center of frozen canine kidney versus time using 7-MHz radiation and recoverable doping material.

SECTION V

CONCLUSIONS

The objective of this initial one-year research effort involving engineering studies of non-ionizing electromagnetic (EM) techniques for thawing deeply-frozen organs was successfully completed. Successful thawing of frozen organs for subsequent transplantation requires that the thawing be achieved rapidly and uniformly throughout the organ. Conventional techniques such as hot-water baths can not produce the required results. Thawing methods that utilize controllable EM fields (as opposed to the uncontrollable EM field configurations and unrepeatable results provided by devices such as microwave ovens) are currently the only promising techniques, and extremely encouraging results have been obtained at Georgia Tech during this initial research effort.

Engineering tasks were performed (1) to investigate the effects of the level of cryoprotectant on the electrical properties (dielectric constant and loss tangent) of both frozen and thawed organ tissues, (2) to derive initial analytical methods for predicting EM field distributions within frozen and thawed kidneys, (3) to design and configure EM illumination systems for thawing frozen rabbit and canine kidneys, and (4) to thaw rabbit and canine kidneys to demonstrate the feasibility of EM single-frequency and multifrequency thawing techniques. The detailed results of these research tasks are fully described in the body of this report, and in addition, specifics of these research investigations have been transmitted to the scientific community via the three publications listed in Section II.

In summary, long term cryopreservation of human organs for subsequent thawing and transplantation offers a hope for improved medical treatment in the future. Use of a multifrequency electromagnetic radiation system with control of illumination patterns and power can produce the uniform and rapid thawing that is required for large organs comparable in size to human kidneys. A detailed knowledge of the electrical properties of the tissue and field patterns within the tissue is necessary to the design of the multifrequency heating system. Moreover, these parameters must be known both as functions of temperature and cryoprotectant level.

Frozen canine kidneys have been thawed uniformly with the equipment and procedures described in this report. The addition of automatic control based on reflected power should increase the rate at which thawing can be safely handled, and additional experiments will show the power versus time schedule that will be required as functions of organ size and shape.

SECTION VI

RECOMMENDATIONS

The results and conclusions of these investigations show that additional research is needed to provide the information necessary to further develop these electromagnetic thawing techniques for use with organs which are comparable in size to human organs. The ultimate goal is to design and develop an electromagnetic thawing system for clinical use. To achieve this eventual goal, it is recommended that engineering methodologies be developed for use in the recovery of cryopreserved canine kidneys. In the development of these methodologies, the following tasks would be performed:

1. Determine the electrical properties of canine kidneys as a function of tissue type in different parts of the kidney, temperature, and frequency of EM radiation,
2. Further develop analytical tools to predict the internal field configuration for two-layered realistic geometrical models of organs for non-planar incident waves,
3. Investigate the use of reflected power as an indicator of organ temperature and phase state,
4. Develop engineering techniques for EM thawing of large organs,
5. Investigate instrumentation techniques for cryogenic temperature measurement in organs in electromagnetic environments,
6. Design and develop an electronic control network for automatic shutoff of the electromagnetic power source(s) based on measured reflected power and temperature,

7. Formulate an engineering methodology for rapid, uniform, hygienic thawing of cryogenically preserved large organs, and
8. Design and develop a prototype model of a clinical EM thawing system and test the system over a period long enough to demonstrate its usefulness.

SECTION VII

REFERENCES

1. Karow, A.M. Jr., Abouna, G.J.M., and Humphries, A.L., Jr., Organ Preservation for Transplantation, Little, Brown, Boston, 1974.

2. Whittinham, D.G., Leibo, S.P., and Mazur, P., "Survival of Mouse Embryos Frozen to -196° and -269°C," Science, Vol. 178, pp. 411-414, 1972.

3. Shimada, K., Asada, M., and Asahina, E., "Electron Microscopic Observation of Ice Crystals Formed in HeLa Cells During Rapid Freezing", Low Temperature Science, Vol, 29B, p. 83, 1971.

4. Asahina, E., Hisada, Y., and Emura, M., "Microscopic Observations of Innocuous Intracellular Freezing in Very Rapidly Cooled Tumor Cells", Low Temperature Science, Vol. 15B, p. 36, 1968.

5. Halasz, N.A., Rosehfield, H.A., Orloff, M.J., and Seifert, L.N., "Whole Organ Preservation. II. Freezing Studies", Surgery, Vol. 61, p. 147, 1967.

6. Lehr, H.B., "Progress in Long-Term Organ Freezing", Transplantation Procedures, Vol. 3, p. 1565, 1971.

7. Rajotte, R.A., Dossetor, J.B., Voss, W.A.G., and Stiller, C.R., "Preservation SLudies on Canine Kidneys Recovered from the Deep Frozen State by Microwave Thawing", Proceedings of the IEEE, Vol. 62, No. 1, January 1974.

8. Burns, C.P., Burdette, E.C., and Karow, A.M., "Thawing of Rabbit Kidneys from -79°C with 2450 MHz Radiation", Abstracts of the Twelfth Annual Meeting of the Society for Cryobiology, August, 1975.

9. Burdette, E.C., and Shoji, M., "Recovery of Frozen Granulocytes Using Microwave Thawing Techniques," Proceedings of the 28th Annual Conference on Engineering in Medicine and Biology, Sept. 1975, pp. 234.

10. Ecker, H.A., Burdette, E.C., and Cain, F.L., "Simultaneous Microwave and High Frequency Thawing of Cryogenically Preserved Canine Kidneys", Record of the 1976 IEEE International Symposium on Electromagnetic Compatability, July 1976.

11. Burns, C.P., Burdette, E.C., and Popovic, V.P., "Electromagnetic Thawing of Frozen Granulocytes", Proceedings of the 1975 Microwave Power Symposium, pp. 30-36, May 1975.

12. Von Hippel, A.R., "Tables of Dielectric Materials," Dielectric Materials and Applications, M.I.T. Press, 1964, pp. 65-69, 84, 85.

13. Daniel, V.V., Dielectric Relaxation, Academic Press, 1967, pp. 154-162.

14. Harvey, A.F., Microwave Engineering, Academic Press, Inc., New York, NY., c. 1963, pp. 233-235, 287-290.

15. Zimmer, R.P., Ecker, H.A., and Popovic, V.P., "Selective Electromagnetic Heating of Tumors in Animals in Deep Hypothermia", IEEE Transactions on Microwave Theory and Techniques, February 1971.

16. Montgomery, C.G., Technique of Microwave Measurements, McGraw-Hill, 1947, Chapter 10.

17. Hasted, J.B., Aqueous Dielectrics, Chapman and Hall, 1973, pp. 56-57.

18. Schwan H.P., "Electrical Properties of Tissues and Cells", Advances in Biological And Medical Physics, Vol. V, J.H. Lawrence and C.A. Tobias, Editors, Academic Press, 1964, pp. 291-425.

19. Shapiro, A.R., Lutomirski, R.F., and Ura, H.T., "Induced Fields and Heating Within a Cranial Structure Irradiated by an Electromagnetic Plane Wave", Transactions on Microwave Theory and Techniques, Vol. 19, No. 2, pp. 187-196, 1971.

20. Ho. H.S, Guy, A.W., Sigelmann, R.A., and Lehmann, J.F., "Microwave Heating of Simulated Human Limbs by Aperture Sources," Transactions on Microwave Theory and Techniques, Vol. 19, No. 2, 1971.

21. Guy, A.W., "Analyses of Electromagnetic Fields Induced in Biological Tissues by Thermographic Studies on Equivalent Phantom Models", Transactions on Microwave Theory and Techniques, Vol. MTT-19, No. 2, pp. 205-215, 1971.

22. Harrington, R.F., Field Computation by Moment Methods, New York, McGraw-Hill, pp. 230-238, 261, 1961.

23. Wu, T.K., and Tsai, L.L., "Numerical Analysis of Electromagnetic Fields in Biological Tissue," Proceedings of the IEEE, Vol. 62, No. 8, August 1974.

24. Discussions and papers presented at Joint U.S. Army/Georgia Institute of Technology, Microwave Dosimetry Workshop, Georgia Institute of Technology, June 1972.

25. Lehmann, P.F., Guy, A.W., Johnston, V.C., Brunner, G.D., and Bell, J.W., "Comparison of Relative Heating Patterns Produced in Tissues by Exposure to Microwave Energy at Frequencies of 2450 and 900 Megacycles", Archives of Physiological and Medical Rehabilitation, Vol. 43, pp. 69-76, February 1962.

26. Burdette, E.C. and Studwell, M.L., "Evaluation of Cryogenic Temperature Sensors for Use in Electromagnetic Fields", Record of the 1976 IEEE International Symposium on Electromagnetic Compatibility, July 1976.

27. Osborne, S.L. and Frederick, J.N., "Microwave Radiations--Heating of Human and Animal Tissues by Means of High Frequency Current with Wavelength of Twelve Centimeters (the Microtherm)", Journal of the American Medical Association, Vol. 137, pp. 1036-1040.

28. Bowman, R.R., "A Temperature Probe for RF Heated Material", Proceedings of the 1975 Microwave Power Symposium, pp. 172-173.

29. Christensen, D.A., "An Optical Etalon Temperature Probe for Biomedical Applications", Proceedings of the 28th Annual Conference on Engineering in Medicine and Biology, Vol. 17, p. 249.

30. Johnson, C.C., Durney, C.H., Lords, J.L, "Liquid Crystal Fiberoptic Temperature Probe for the Measurement of Electromagnetic Power Absorption in Tissue", 1974 IEEE S-MTT International Microwave Symposium Digest, pp. 32-34.

31. Burns, C.P., and Burdette, E.C., "Thawing of Frozen Organs with Electromagnetic Radiation", Proceedings of the IEEE 1974 Region 3 Conference, April 1974.